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A MORPHOLOGICAL AND BIOLOGICAL STUDY OF THE TREMATODE, *SELLACOTYLE MUSTELAE* N. G., N. SP.[1]

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I. INTRODUCTION

Sellacotyle mustelae is a minute trematode parasite found in nature in the mink (*Mustela vison*) but infective to many carnivores. The worms are imbedded in the mucosa of the small intestine and being only one quarter to one half a millimeter in length are almost invisible to the naked eye, and are found in intestinal scrapings with the aid of the dissecting microscope.

This species was found occasionally during the years 1929 to 1931 in wild and captive mink in Minnesota. During the winter of 1932, a heavy incidence of this parasite was found among the mink of a fur farm in Northern Minnesota, approximately 25 per cent of the four thousand animals being infected. As the abundance of material seemed to offer an excellent opportunity to find the source of infection and thus to trace the life history of a parasite of the mink, an animal of growing economic importance, the study discussed in the following pages was undertaken.

II. ACKNOWLEDGMENTS

It is a pleasure to express my appreciation to Dr. W. A. Riley for his guidance and criticism during the course of this work. I am indebted to Mr. E. M. Godfrey, of Deer River, Minnesota, for the fullest co-operation in providing material and to the Champion Animal Food Company of Minneapolis for furnishing many experimental animals. I wish to thank Dr. E. W. Price for examining specimens of the adult fluke, Dr. F. C. Baker for identifying the snail host, and Dr. B. T. Simms for furnishing specimens of *Nanophyetus salmincola* for comparative study.

III. TECHNIQUE

Living specimens, whole mounts stained in borax carmine, Ehrlich's acid hematoxylin, or alum cochineal, and serial sections were used in

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working out the morphology of the adult flukes. Because of the thickness of the specimens and the density of the vitellaria the detailed morphology was studied almost entirely in serial sections. Sagittal sections were found most useful but frontal and transverse sections were made also. Living specimens were studied for certain phases of the reproductive and excretory systems. The latter system is more easily studied in young forms from the mammalian host before the maturity of the reproductive organs. The measurements of the entire worm, suckers, testis and ovary are averages taken from twenty-five representative whole mounts. These are in the state of contraction of worms dead or

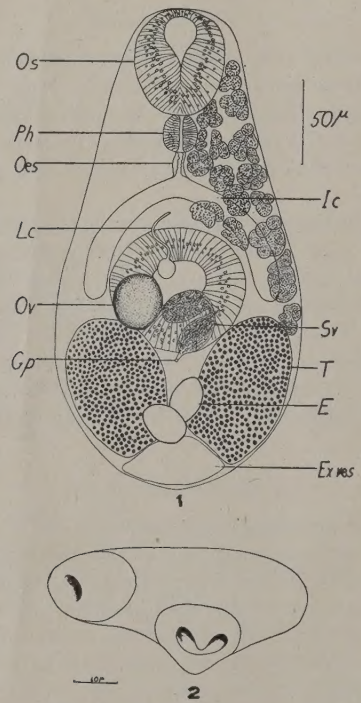


FIG. 1. Adult *Sellacotyle mustelae,* dorsal view.

E	Egg in uterus.	O s	Oral sucker.
Ex ves	Excretory vesicle.	Ov	Ovary.
I c	Intestinal ceca.	Ph	Pharynx.
G p	Genital pore.	S v	Seminal vesicle.
L c	Laurer's canal.	T	Testis.
Oes	Oesophagus.		

FIG. 2. Adult *Sellacotyle mustelae,* side view, showing the ventral projections of the acetabulum.

at rest but living worms may extend the anterior part of the body so that the length is greater than that given. Measurements of other organs are taken from the most favorable fifteen series of section. It must be pointed out that in such plastic animals as trematodes the relative positions of many organs are subject to variations because of changes in the shape of the worm. For example, the seminal receptacle of this species is at times anterior and at times posterior to the ovary.

IV. DIAGNOSIS AND SYSTEMATIC POSITION

Detailed morphological studies make it evident that this minute trematode from the mink not only represents a new species, but must be regarded as the type of a new genus.

Sellacotyle n. g.

(Text figures 1–4)

Generic Diagnosis.—Troglotremidae. Small pyriform distomes with conspicuous suckers. Testes ovoid and symmetrically arranged in the posterior part of the body. Ovary spherical or ovoid, above the acetabulum. Uterus sac-like, between the testes. Seminal receptacle and Laurer's canal present. Seminal vesicle separated into two parts by a constriction. No cirrus pouch. Genital pore median and close behind the acetabulum. Excretory vesicle sac-like and slightly bicornuate. Cercariae microcercous with a compact group of mucous glands opening on the ventral side near the posterior end. Flame cell formula of cercaria 2(2+2+2). Type and only known species—*Sellacotyle mustelae.*

Sellacotyle mustelae n. sp.

Specific Diagnosis.—Sellacotyle. Body pyriform, circular in cross section 335 μ long by 190 μ wide. Oral sucker at anterior end of body and directed anteroventrad 100 μ in diameter. Acetabulum slightly larger with its center just behind the middle of the body. Each side of the acetabulum forms a conical, ventral projection of such form that the entire organ resembles an inverted saddle. Entire body except the suckers covered with minute spines about 2 μ long. Prepharynx 5 μ long. Pharynx sub-spherical, 32 μ x 36 μ followed by the oesophagus 30 μ–40 μ long. Digestive ceca extend beyond the center of the acetabulum. Vitellaria consist of relatively large follicles distributed dorsad from the oral sucker to the testes, and extending low on the sides between the suckers. Ovary spherical or ovoid 30 μ–40 μ in diameter, median or slightly to one side or the other above the acetabulum. Seminal receptacle 15 μ–20 μ in diameter, dorsal or dorsal and anterior to ovary. Laurer's canal about 50 μ long, posterior to receptacle. Testes ovoid, 100 μ x 65 μ side by side in the posterior end of the body. Uterus sac-like, between testes and containing one to five eggs. Metraterm extends from uterus to genital pore which is on the median ventral line behind the acetabulum. Eggs operculate 60 μ long by 54 μ wide, undeveloped when deposited. Seminal vesicle in two parts, lying free in the parenchyma, close behind and above the acetabulum. Male genital duct opens into the metraterm from above.

Hosts.—Primary, Mink (*Mustela vison*), and, as indicated by experimental feedings, various other piscivorous mammals. First intermediate, *Campeloma rufum*; second, *Ameiurus melas* ("bull-heads"), *Catostomus commersonii, Umbra limi, Esox lucius, Perca flavescens, Notropis* sp.

Location.—Small intestine.

Distribution.—North America (United States, Minn., Mich., Conn.(?), Ontario.

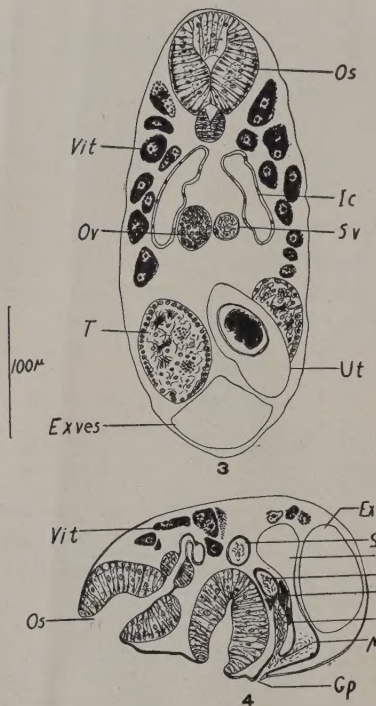


FIG. 3. Camera lucida drawing of a frontal section of an extended specimen.

FIG. 4. Camera lucida drawing of a sagittal section of a contracted specimen.

Ex ves	Excretory vesicle.	P g	Prostatic glands.
G p	Genital pore.	P p	Pars prostatica.
I c	Intestinal ceca.	S v	Seminal vesicle.
Met	Metraterm.	T	Testis.
O s	Oral sucker.	Ut	Uterus.
Ov	Ovary.	Vit	Vitellaria.

Type specimens.—United States National Museum Helminthological Collections No. 8649; *cercaria,* No. 8651. Paratypes in the collection of the Division of Entomology and Economic Zoology, Univ. of Minn., and of the author.

Sellacotyle mustelae belongs in the family *Troglotremidae* Odhner (1914) of the superfamily *Troglotrematoidea* (Faust, 1929). This family was established by Odhner to include the genera *Pholeter* (Odhner, 1914). *Collyriclum* (Kossack, 1911), *Renicola* (Cohn, 1904), *Paragonimus* (Braun, 1899) and *Troglotrema* (Odhner, 1914). His characterization of the family is as follows: "More or less flattened 'Distomes' or 'Monostomes' of rounded form, 2–13 mm. long. Posterior end

drawn out into a small 'tail.' Venter flat or slightly concave. Dorsum arched. Skin covered with spines. Musculature of those living in cysts weakly developed, even in the suckers. Digestive tract with pharynx, oesophagus and intestinal ceca which end more or less near the posterior end. Excretory vesicle Y-shaped or sac-like. Genital pore close before or behind the acetabulum—median or slightly to the left. Cirrus pouch usually lacking (except in *Troglotrema*). Pars prostatica and seminal vesicle always present. Testes symmetrical, longitudinally disposed, in or behind the middle of the body. Ovary immediately in front of the testes on the right—usually lobed (except in *Troglotrema*). Receptaculum seminis and Laurer's canal present. Yolk glands usually very well developed and entirely or chiefly distributed under the dorsal surface, only a median line being left open. Uterus long and loosely coiled or shorter and knotted. Eggs in the first case small, 17 μ–25 μ long, in the latter case considerably larger, 63 μ–85 μ and according to some statements up to 120 long. Parasites of carnivores or birds, usually paired in cyst like cavities."

Braun-Seifert (1915) give the family name on page 164 as *Troglotremidae* but on page 184 as *Troglotrematidae*. The latter form has been followed by most subsequent authors. As the spelling appears to be inadvertent, the present writer sees no reason for using it in place of the original spelling of Odhner.

Baer (1931b and 1932) added the genus *Nephrotrema* to this family. *Nephrotrema truncatum* (Leuckart, 1842) Baer, 1931, is a distome 3 mm. to 5 mm. in length found in the kidneys of *Neomys fodiens* and *Talpa europaea* in Switzerland. The body is thick, spined, and tapers to a point at the posterior end. The intestinal ceca separate immediately behind the pharynx and extend the entire length of the body. The ovoid testes are side by side anterior to the acetabulum while the spherical ovary is posterior to it. The genital pore is posterior to the acetabulum. The relative positions of the testes and ovary are the reverse of their positions in the other members of the family. Nevertheless all the other morphological characters are in agreement with the family diagnosis. The most significant similarity is the habit of migrating into the tissue of some organ outside the alimentary tract. *Paragonimus* ordinarily is found in cysts in the lungs of mammals, *Collyriclum* in subcutaneous cysts in birds, *Pholeter* in cysts in the stomach wall of marine mammals, *Renicola* in the kidneys of birds, and *Nephrotrema* in the kidneys of mammals. Thus this common biological characteristic, as well as the morphological similarity shows a close relationship between these forms.

On the basis of the relative positions of the testes and ovary Baer (1931b) separated the *Troglotremidae* into two subfamilies. The *Nephrotrematinae* include the genus *Nephrotrema* or forms with the

testes anterior to the ovary. The *Troglotrematinae* include the other five genera or forms with the testes posterior to the ovary.

The salmon poisoning fluke of dogs, *Nanophyetus salmincola* (Chapin, 1926) Chapin in Hall, 1927, is an intestinal parasite of carnivores showing a close relationship to *Sellacotyle mustelae*. Witenberg (1932) redescribed this species and placed it in the genus *Troglotrema*.

According to his description the salmon poisoning fluke is similar to *Sellacotyle mustelae* in its general features but differs from it in the following particulars: It is larger, measuring 0.8 to 1.1 mm. in length. It is provided with a well-developed cirrus pouch while *Sellacotyle* has none. The intestinal ceca pass around the acetabulum and between the testes while those of *Sellacotyle* reach only to the middle of the acetabulum.

Although both Witenberg and Chapin failed to find a seminal receptacle in *Nanophyetus salmincola,* the author has found this organ in several specimens sectioned serially. It is spherical, 15 μ to 25 μ in diameter and above the acetabulum.

The salmon poisoning fluke agrees, except for size, with the family diagnosis of the *Troglotremidae* but can not well fit in the genus *Troglotrema*. According to Baer (1931a) and Odhner (1914), as well as the original description (Leuckart, 1842) *Troglotrema acutum,* the type and only known species possesses a cirrus while *Nanophyetus salmincola* has none. Furthermore *Troglotrema acutum* inhabits the frontal sinuses of mustelids while *Nanophyetus salmincola* is found in the intestinal tract of carnivores. These differences, the author believes, exclude the salmon poisoning fluke from the genus *Troglotrema*.

Sellacotyle mustelae appears, on the basis of morphological and biological features, to be closely related to *Nanophyetus salmincola*. Both are small pyriform distomes. The arrangement of the suckers, intestinal ceca, testes, ovary, seminal vesicle, genital pore, and vitellaria are similar in both species. It is worthy of note that in both cases the seminal vesicle is divided into an upper and a lower part. The cercaria of *Nanophyetus salmincola* (Simms, Donham, and Shaw, 1931, and Sinitsin, 1929) is a microcercous form possessing the mucous gland described for *Sellacotyle*. It has no stylet. It develops in the operculate snail, *Goniabasis plicifera silcula* Gould, and encysts in fish of the family *Salmonidae*.

These points, together with the differences noted above, indicate that these two species of flukes belong to separate but closely related genera.

On first consideration, it appears unreasonable to place these two genera of small, intestinal flukes in a family of larger cyst inhabiting forms such as *Paragonimus* and *Troglotrema*. Nevertheless, apart from the discrepancy in size *Nanophyetus* and *Sellacotyle* agree morphologically with the family diagnosis. Furthermore, the similarity of the

microcercous cercariae of *Nanophyetus, Sellacotyle,* and *Paragonimus* shows a relationship between these genera.

For the inclusion of the genera *Nephrotrema, Sellacotyle,* and *Nanophyetus* Odhner's family diagnosis of the *Troglotremidae* may be modified as follows:

REVISED DIAGNOSIS OF TROGLOTREMIDAE

Thick monostomes or distomes 2 to 13 mm. in length living in cyst-like cavities in birds or mammals or smaller pyriform distomes living in the intestine of mammals. Skin covered with spines. Digestive tract with pharynx, oesophagus and two intestinal ceca of varying length. Excretory vesicle Y-shaped or sac-like. Genital pore with no external modification, close before or behind the acetabulum. Seminal vesicle large, cirrus pouch absent except in *Troglotrema.* Testes equal and side by side, anterior or posterior to the acetabulum. Ovary anterior to testes except (*Nephrotrema*) when the latter are anterior to the acetabulum. Seminal receptacle and Laurer's canal present. Uterus long and loosely coiled with eggs small, 17 μ to 25 μ in length or uterus short and eggs larger, 60 μ to 85 μ in length. Cercariae of the microcercous type.

It seems desirable to separate the genera *Nanophyetus* and *Sellacotyle* from the other genera of the family. Therefore the author proposes the new subfamily, *Nanophyetinae.* This group is characterized as follows:

DIAGNOSIS OF NANOPHYETINAE

Troglotremidae. Small pyriform distomes less than 1.1 mm. in length, found in the intestine of carnivores. Seminal vesicle large and divided into two parts. Cirrus pouch present or absent, cirrus absent. Type genus, *Nanophyetus* Chapin in Hall, 1927.

A key separating the subfamilies of the *Troglotremidae* follows:

1 (4) Large thick monostomes or distomes living in cystic cavities in birds or mammals.—2.

2 (3) Testes posterior to acetabulum and ovary—subfamily *Troglotrematinae* (Baer, 1931).

3 Testes anterior to acetabulum and ovary—subfamily *Nephrotrematinae* (Baer, 1931).

4 Small piriform flukes less than 1.1 mm. in length found in the intestine of carnivores—subfamily *Nanophyetinae* new subfamily.

The two genera of the *Nanophyetinae* are separated as follows:

1 Length more than 0.8 mm., intestinal ceca passing between testes. Cirrus pouch present.—*Nanophyetus* Chapin (1926).

2 Length less than 0.5 mm., intestinal ceca not passing posterior to the acetabulum. Cirrus pouch absent—*Sellacotyle* (n. g.).

Macroorchis spinulosus Goto in Ando, 1919, appears to belong in the family *Troglotremidae* as pointed out by Witenberg (1932). This parasite developed in white rats which had been fed metacercariae from the crab, *Potamon dehaani,* in Japan. Unfortunately the description is not sufficiently detailed to permit a final judgment as to its systematic position. The figure (Dollfus, 1925) shows a distome fluke with the intestinal ceca reaching to the posterior end of the body. The testes are unlobed and outside the ceca. The uterus and ovary are between the testes.

The details of the male genital duct and the genital pore are not shown. The size is not given but as Dollfus says the adult differs little from the metacercaria, it is probably a small form. These features together with the fact that the worm was found in the intestine of the experimental host suggest that *Macroorchis spinulosus* may belong in the *Nanophyetinae*.

Odhner (1914, p. 244) calls attention to the close relationship between the *Troglotremidae* and the *Heterophyidae*. *Nanophyetus* was first ascribed, as *Nanophyes*, to the latter family by Chapin (1926) and indeed in its general morphology and type of life history, encysting in fish, appears to belong there. However, in possessing a cirrus pouch and no external modifications of the genital pore, it does not fit the family diagnosis (Witenberg, 1929, and Ransom, 1920). Furthermore, the cercaria of *Nanophyetus* is short-tailed like that of *Paragonimus* while the known cercariae of heterophyids are lophocercous forms with eye spots. *Sellacotyle mustelae* even more resembles the *Heterophyidae* (Faust and Nishigori, 1926) in that it possesses no cirrus pouch but again the unmodified genital pore and the short-tailed cercaria place it in the *Troglotremidae*.

Thus the *Nanophyetinae* emphasize the close relationship between the *Heterophyidae* and the *Troglotremidae* and appear to show a transition between them.

V. INTERMEDIATE HOSTS

The source of infection of the mink of the fur farm mentioned in the introduction was found to be the fish, *Ameiurus melas*, which harbored the metacercariae of *Sellacotyle mustelae*. These fish from nearby lakes and streams formed a substantial part of the diet of the mink and thus afforded ample opportunity for the completion of the life cycle of the parasite. Subsequently a wide variety of other fish from the same locality were found to be infected. The cercariae were found in the snail, *Campeloma rufum*, from a stream in which the fish were heavily infected. (Pl. I, Fig. 1).

The first intermediate host, *Campeloma rufum*, is a large thick shelled operculate snail. It is primarily a stream species living in the mud and débris under the banks of small streams frequented by wild mink.

VI. CERCARIA

For microscopic study the cercariae were taken after naturally emerging or were dissected from the snail. During the latter part of the summer and the fall, those found by crushing the snail were indistinguishable from those which naturally emerged. Living specimens, unstained or stained lightly with neutral red, show practically all the internal

organs better than fixed material. However, a number of features, but particularly the genital anlage, are best seen in total mounts. These were stained in borax carmine, Ehrlich's acid hematoxylin or alum cochineal. The finer excretory tubules and flame cells can, of course, be studied only in living specimens. Measurements were taken from twenty specimens fixed in warm formalin and placed under the cover glass. This technique, used with care, did not distort the specimens.

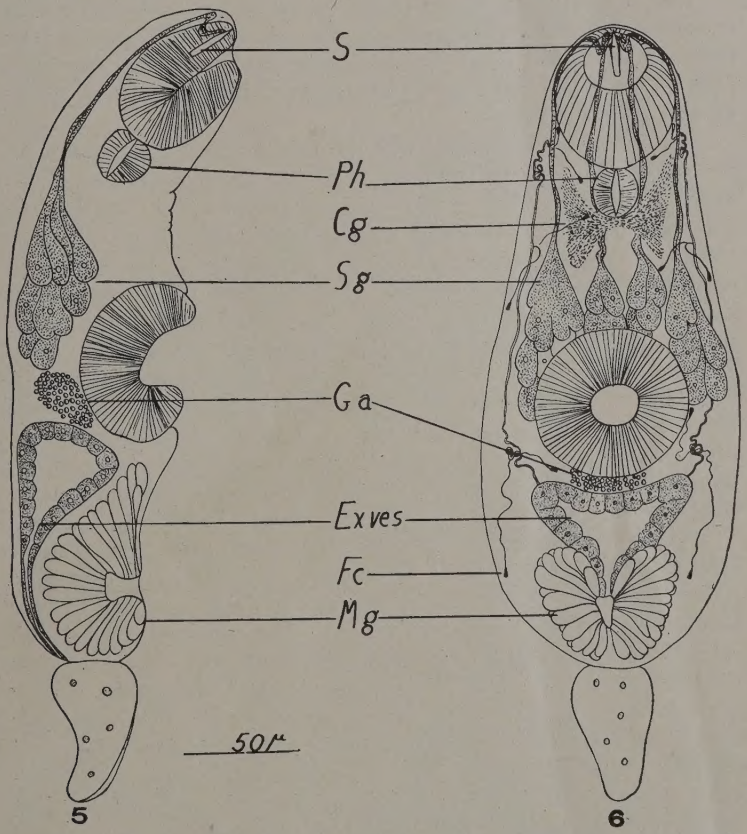


FIG. 5. Camera lucida drawing of the cercaria of *Sellacotyle mustelae*, side view.

FIG. 6. Camera lucida drawing of the cercaria of *Sellacotyle mustelae*, ventral view.

C g	Cerebral ganglia.	M g	Mucous gland.
Ex ves	Excretory vesicle.	Ph	Pharynx.
F c	Flame cell.	S	Stylet.
G a	Genital anlage.	S g	Stylet glands.

The cercariae of *Sellacotyle mustelae* (Text figures 5 and 6 and Plate I, Figure 3) average 260 μ in length and 105 μ in width. The tail is 60 μ long by 39 μ wide. The body is practically circular in cross section. It is very plastic and can extend to a much greater length than the above measurements, taken from specimens in the resting position, indicate. When floating at rest in water, the body is slightly flexed ventrad and is not extended in length. The tail is pyriform and slightly concave so there is a shallow longitudinal groove on the ventral surface. The entire body is covered with minute backward pointing spines. These are especially noticeable on the tail where they are longer than on the body but never form conspicuous bristles as in *Paragonimus* (Ameel, 1932) or *Nanophyetus* (Sinitsin, 1929).

The oral sucker is terminal with the opening directed anteroventrad. It measures 56 μ in length and 52 μ in width. In the dorsal part of the sucker is the stylet which is 18 μ long and provided with lateral shoulders about one-third of its length from the point. Behind the oral sucker there is a spherical pharynx about 17 μ in diameter. This is the only part of the digestive tract visible. No prepharynx can be seen. The acetabulum is 52 μ wide by 47 μ long. The ventral projections from its sides which were described for the adult are slightly developed.

Immediately behind the pharynx lies the "brain" or cerebral ganglia. This mass of nervous tissue is constricted in the middle and fan-shaped laterad so that it looks like a butterfly. It is faintly visible as an opaque white mass in living specimens and appears as a clear space in specimens stained with alum cochineal. In stained specimens the nuclei of the surrounding tissue seem to be more numerous about its periphery.

There are a number of unicellular stylet glands arranged in a dense mass above and anterior to the acetabulum. It is extremely difficult to count these glands as they can be seen only in living specimens and are very delicate. There are at least sixteen. The cytoplasm is filled with refractive granules that stain intensely with neutral red. From the mass of stylet glands four trunks of ducts pass forward to the stylet. There are four ducts in each trunk, making sixteen in all. The two outer trunks pass from the lateral portions of the gland mass forward, around the sides of the oral sucker. The two median trunks pass directly forward, dorsad from the brain and dorsad from the oral sucker. Just before reaching the stylet each trunk bends back sharply on itself and then forward again making an S curve. The courses of the four trunks are particularly plain in specimens flattened under the cover glass but the presence of four separate and distinct trunks can be clearly seen in unflattened specimens in their normal position.

Just behind the ventral sucker is a mass of cells with distinct, deeply staining nuclei, the genital anlage. This shows no differentiation into the various organs.

The excretory vesicle is a large thick-walled sac which opens dorsad from the tail at the posterior end of the body. It is somewhat heart shaped being wide in front and tapering toward the posterior end. Its wall is about 7 μ thick and consists of a cuboidal epithelium, the nuclei of whose cells are larger and less dense than the other nuclei of the cercaria. It is best seen in living specimens but shows in specimens stained with alum cochineal.

From the antero-lateral corners of this sac a collecting duct passes forward on each side. Just anteriorly from the excretory sac it is tangled and convoluted. In this region two small ducts are given off, one to a flame cell in the posterior part of the body and one to a flame cell near the mid line. Two more flame cell ducts are given off just in front of the acetabulum. Near the oral sucker the collecting tubule again is convoluted and finally branches into two flame cell ducts. The flame cell formula is $2(2+2+2)$. The minute unicellular cystogenous glands are peripherally arranged in the parenchyma.

Ventrad from the posterior portion of the excretory sac there lies a cluster of unicellular glands which open through a common ventral aperture. From the ventral side this cluster appears heart-shaped. The slit-like opening, about 20 μ from the posterior end of the body is about in the middle of the gland. From the side the mass appears semicircular. The gland stains deeply with hematoxylin. It takes up neutral red, becoming an orange yellow.

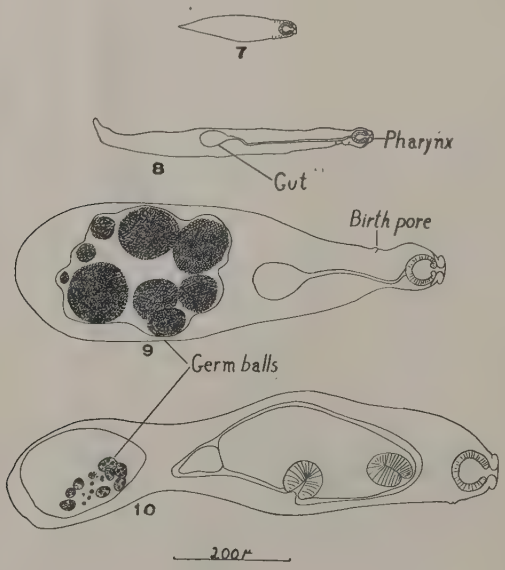
A similar gland is described for *Cercaria trigonura* Cort (1914 and 1915) and is figured by Sinitsin (1929) for the cercaria of *Nanophyetus salmincola*. In neither case was the function ascertained, but an adhesive product was suggested. In *Sellacotyle mustelae* this gland produces a mucous secretion which keeps the cercariae entangled together and floating in the water, or causes them to adhere in masses to solid objects. When these cercariae are shed by a snail, a large number are found suspended in the water in a stringy mucous substance. It is difficult to pipette out one or a few as the whole mass coheres. The substance is produced by the cercariae themselves for if a number are dissected from a snail and stirred up in clean water, in a short time they are entangled in the mucus as are those naturally shed from the snail. The gland just described stains intensely with Mayer's muci-carmine, a specific stain for mucin.

The cercaria of *Sellacotyle mustelae* bears a close resemblance to *Cercaria trigonura* Cort (1914 and 1915). This species was found in the snail *Campeloma decisum* (called *C. subsolidum* in the original publication but later identified as *C. decisum* according to Ward and Whipple, 1918, and personal correspondence from Dr. W. W. Cort) from Hartford, Connecticut. The cercaria of *S. mustelae* agrees in its gen-

eral features with Cort's description. *C. trigonura* is 240 μ long and 60 μ wide and its tail is 52 μ long and 24 μ wide. Thus the two forms easily fall within the size range of one species. The suckers, stylet, excretory sac, genital anlage and mucous gland appear to be identical in the two forms. As Cort's description was made largely from preserved material the flame cells are not figured. Although his species is described as spined only over the anterior end the absence of spines from the rest of the body might well be due to preservation.

One difference, however, seems to be significant. In *C. trigonura* the ducts from the stylet glands pass forward as one median trunk while in the cercaria of *Sellacotyle mustelae* they are in four trunks. This difference, the author believes, indicates that the two cercariae are separate species. The possibility remains, however, that a restudy of *Cercaria trigonura* from the type locality may show it to be identical with the cercaria of *Sellacotyle mustelae* in which case the specific name of the latter would be supplanted by that of the former.

The season of cercaria production is the late summer and fall, the first cercaria having been found July 25th, while before that time, only undeveloped forms were found in the infected snails. In the laboratory, the maximum cercaria production occurred during August and the first part of September, but occasional emergences were noted until the middle of January.



FIGS. 7–10. Rediae of *Sellacotyle mustelae*.

In the snail, the cercariae are found free in the tissues. They are particularly numerous in the walls of the uterus which they distend until the organ appears white and turgid in contrast to its normal dark, flaccid condition.

The rediae (Text figures 7, 8, 9, and 10) are found mingled with the cercariae. They vary greatly in size, the length being 200 μ to 830 μ and the width 50 μ to 160 μ. The general form is cylindrical but the smallest ones taper from the anterior end to a point at the posterior end, while the larger ones are rounded. The larger radiae when full of cercariae are ovoid but after the season of cercaria production are irregularly cylindrical and constricted in one or more places.

A spherical pharynx at the anterior end leads into the blind, cylindrical gut. Developing cercariae are found only in the larger rediae, that is those more than about 400 μ in length. The spherical germ balls are formed in the posterior end while the cercariae are found farther forward. The birth pore opens on the side just posterior to the pharynx. From one to eight cercariae are found in each redia. The cercariae escape from the birth pore of the rediae and lie free in the tissues of the snail from which they emerge periodically in large numbers.

In snails known to have been infected previously, which were dissected during the winter months, there were few cercariae but many rediae which appeared shriveled and degenerating. There were, in addition, some small pointed rediae which were not producing cercariae. This may indicate that the small rediae remain over winter in the snail to produce cercariae the second year.

No stages other than the rediae and cercariae were found in the snails during the period of this study.

VII. INFECTION OF THE FISH HOST

The cerceriae of *Sellacotyle mustelae* readily penetrated and encysted in all the species of fish which were tried in the laboratory. These included *Ameiurus melas, Schilbeodes byrinus, Moxostoma aureolum, Pimephales promelas, Semotilus* sp. and *Notropis* sp. As these fish and the fish in which the fluke cysts were found in nature include representatives of a number of widely separated families it appears that *Sellacotyle mustelae* at this stage has little host specificity and can utilize any of our species of fresh water fish as second intermediate host.

Young *A. melas* were most readily infected. This fact together with their availability in large numbers and almost complete freedom from internal parasites made them very convenient laboratory animals.

In many instances the fish were exposed to cercariae by placing them in a jar with an infected snail. In the first experiments the fish were killed by the large number of cercariae. July 30 the three jars showing

the largest number of cercariae were selected and two small bull heads about 1¾ inches in length were placed in each. The same number of fish were placed in three jars with snails but no cercariae as controls. In the jars with cercariae the fish died in about seven hours while the controls remained unaffected. The muscles of the dead fish, but especially those at the base of the tail, the bases of all the fins and the tissue under the skin of the head teemed with actively moving and encysted cercariae.

The estimated number of cercariae shed at one time varied from a few hundred to fifteen thousand. It was found that fish placed in moderately infested jars became infected but would live almost indefinitely. The method of diluting the cercariae in a larger container and exposing a number of fish was also successful. The larger fish appeared to be better able to survive severe exposure. Although no quantitative analysis was made of the number of cercariae tolerated by a fish, experience soon enabled the author to strike such a ratio between fish size and number of cercariae that the fish would survive and the parasites develop normally. Apparently the effect of the cercariae on the fish was an all or none phenomenon, the fish either dying within a day after exposure or surviving indefinitely.

An attempt was made to determine the time of exposure to cercariae necessary to kill the fish. August 2, 1932, six snails were found shedding large numbers of cercariae. The water from the six jars was poured into a pan and ten small fish were put in. Two fish were removed after each of the periods: 10, 20, 30, 40, and 50 minutes. Those exposed 10 and 20 minutes survived, and on subsequent examination showed a few encysted cercariae. Those exposed 30 minutes or longer were killed by the cercariae.

The cercariae can be watched as they penetrate the fish. A small bull head is held in a dish of water under the dissecting microscope. A large number of cercariae are pipetted onto its surface. Most of the cercariae move about actively by means of their suckers over the surface of the fish, but occasionally one remains with its oral sucker anchored in one place and the rest of the body bending to one side or the other. It gradually disappears into the skin, the whole process consuming from ten to twenty minutes. Many of the cercariae lose their tails before attempting penetration, but in all cases this organ is lost before the parasite is completely within the fish.

Sections of the fish skin during penetration of the cercariae were made to study the process in greater detail. (Plate II, figures 1 and 2). Small pieces with the skin, were cut from freshly killed bull heads and exposed to many cercariae in a watch glass. After varying periods of exposure they were fixed in Bouin's fluid and sectioned perpendicularly to the surface. Those pieces exposed an hour or more showed most cer-

cariae in the act of penetrating the skin. A study of many sections indicates that a cercaria penetrates the epithelium perpendicularly, turns when it strikes the dense connective tissue of the dermis, moves along this a short distance and again turns perpendicularly to the surface to work into the underlying muscles. After the anterior end has penetrated, a purchase in addition to that furnished by the oral sucker, is obtained by the enlargement of the imbedded tip. The actions of the cercariae observed under the compound microscope indicate that the stylet plays an important part in penetration. The sucker attaches itself to a solid object and the stylet on its dorsal side projects forward rhythmically, probing any object it encounters. The part played by the stylet glands has not been determined but the appearance of the cercariae in the sections of fish skin suggests that their secretion has a lytic effect.

The above description gives the mode of penetration into the bullhead, a fish whose integument consists of a cellular epithelium. Sections were similarly made of *Notropis* sp., a scaled minnow, exposed to cercariae. In this case the sections showed the parasites between the scales but none in the act of penetrating them. This indicates that the cercariae work under the scales rather than penetrate them directly, a fact that may account for the larger number of metacercariae in *Ameiurus*.

In tap water the cercariae occasionally encysted but this was never a regular occurrence. However, the formation of cysts occurs when the cercariae are placed in the fluid extracted from the muscle tissue of the bull head. A small bit of muscle is squeezed between two slides and removed with forceps. Cercariae with as little water as possible are placed in the drop of fluid on one of the slides and a cover glass is added. The first sign of cyst formation is seen with the microscope in about ten minutes. The worms cease their active "measuring worm" progression but continue to extend and contract. A thin hyalin sheath appears closely adhering to the surface of the worm. After this surrounds the entire body it loosens so that the worm is free to move inside it. Finally, when the cyst is completely loosened from the cercaria it becomes spherical or ovate from the pressure of the worm inside.

In the fish host the cercariae begin to encyst a few minutes after their penetration. After four hours only a few unencysted worms are found. When first formed the cysts are unattached to the surrounding tissue and fall out readily when the fish is torn to pieces under the dissecting microscope. Later the fish forms a dense fibrous capsule around the cyst. Plate II, figure 3, shows such a capsule twenty-five days after infection. In naturally infected fish, these capsules are very tough and often two to twenty or more cysts may be held in a cluster by the fibrous envelope.

There are a number of morphological changes in the larvae after encystment. The cercarial organs; stylet, stylet glands and mucous gland disappear while the digestive and reproductive systems develop. The mucous gland disappears in the first few hours after encystment. After two or three days the stylet glands have disappeared. The stylet diminishes in size until it finally disappears but it does not drop off entirely. In the cercaria the stylet is 18 μ in length. Four days after encystment in the fish, the stylet is about 10 μ in length. Ten days after encystment it is 4 μ in length and fifteen days after encystment it has completely disappeared.

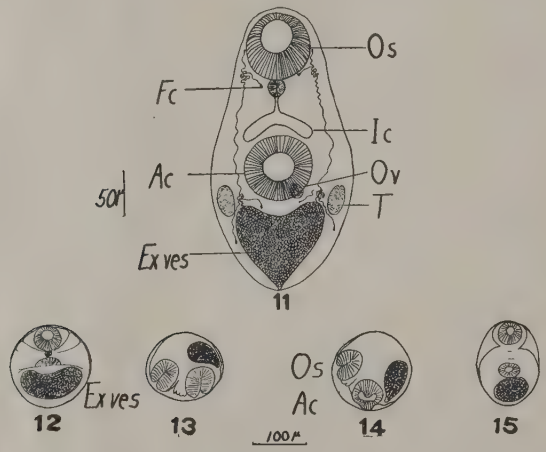


FIG. 11. Metacercaria of *Sellacotyle mustelae* removed from cyst.
FIGS. 12, 13, 14, and 15. Encysted metacercariae in various positions.

Ac	Acetabulum.	O s	Oral sucker.
Ex ves	Excretory vesicle.	Ov	Ovary
Fc	Flame cell	T	Testis.
I c	Intestinal ceca.		

VIII. THE METACERCARIA

In laboratory infected fish the metacercariae or encysted cercariae of *Sellacotyle mustelae* are found throughout the entire body with the exception of the organs of the body cavity. Some of these fish were digested in artificial gastric juice and the number of cysts freed was estimated to be between three and four thousand. They are most numerous in the fins and under the skin of the head but are found in all the muscles. A few cysts are found on the mesenteries but none in the organs of the body cavity or the kidneys. A few are on the gill arches but none on the gills themselves.

In naturally infected fish the cysts are scattered sparsely in the muscles, being most numerous along the course of blood vessels and just under the peritoneum. Larger numbers are found in the adipose and fibrous tissues at the bases of the fins. They are found in masses in the tissues in which the fin rays are inbedded and out into the fins, between the rays, for a distance of 4 or 5 mm. The greatest concentration of cysts is found at the bases of the barbules in *Ameiurus* (Plate II, figure 4). Here the tissue under the skin is practically a solid mass of cysts. In *Ameiurus* there are no cysts in the small adipose dorsal fin just anterior to the tail. Apparently the greatest number of cysts is found in places where there is much surface in proportion to the amount of underlying tissue and where there is a good blood supply.

The smooth clear cyst wall is 2 μ in thickness. The cysts are spherical or, more usually, ovoid measuring from 132 μ to 168 μ (average 149 μ) in length and 122 μ to 142 μ (average 129 μ) in width. The larval flukes inside which differ little in size from the cercariae, fill the cysts completely. They lie in various positions in the cysts and move frequently. (Text figures 12, 13, 14, and 15). Usually the larva is contracted so that the oral and ventral suckers almost touch. Sometimes the anterior end is elongated and doubled back on the rest of the body.

The most conspicuous feature of the encysted fluke is the excretory bladder. This being filled with highly refractive droplets appears black with transmitted or white with reflected light. It usually is more or less reniform. The two suckers are easily seen and often the central projections of the acetabulum, described for the adult, can be identified. The entire larva is covered with minute spines.

The larvae can be dissected from the cysts with difficulty and those thus obtained are often injured. They are more easily obtained by the use of artificial pancreatic juice. The cysts are placed in a 0.1 per cent sodium bicarbonate solution to which a small experimentally determined amount of trypsin is added. After an hour or two at 37° C. many of the larvae have broken out of their csyts. Text figure 11 shows the excysted metacercaria. The excretory system is as in the cercaria except that the bladder is distended with fluid. The flame cells and collecting tubules are unchanged from their condition in the cercaria. Although all the parts of the adult digestive system are differentiated the intestinal ceca have not attained their entire length. The ovary and testes, though small, are found in the same positions as in the adult.

IX. INFECTION OF THE MAMMALIAN HOST AND PATHOLOGY

Cysts from the naturally infected bull heads matured in various mammals to which they were fed. Approximately sixty *Ameiurus melas* were netted from a stream, brought to the laboratory alive and kept in a

tank to be used as needed. The following animals were successfully infected by feeding these fish: ten cats, eight mink, five dogs, six rats, two ferrets, one fox, one racoon and one skunk. In addition a number of *Perca flavescens, Catostomus commersonnii,* and *Umbra limi* were brought to the laboratory on ice. Cysts from each of these species matured in cats.

No difference in the susceptibility to the parasite was noted in these various hosts as the worms developed in maturity in approximately the same time and remained in the host in each case until the autopsy some weeks after infection. Two guinea pigs, and two domestic ducks were forcibly fed the barbules and fins of several heavily infected fish, but no evidence of infection could be found. Fecal examinations of the animals were made daily for two weeks after feeding the infective material. The guinea pigs were autopsied twenty-one and thirty days respectively after feeding but no parasites were found. The two ducks were autopsied thirty-two and forty-three days respectively after feeding and were likewise negative.

In the mammalian host *Sellacotyle mustelae* reaches maturity five days after the infective cysts are ingested. After this period eggs appear in the feces of the host and mature flukes can be found in the intestine. There is some spread in the time of development, however, and immature flukes with no eggs in the uterus can be found as late as eight days after feeding.

In addition to the above summarized feeding experiments with naturally infected fish, small bull heads infected with cercariae in the laboratory by the above described methods, were fed to cats, rats, and mink.

The first trials were unsuccessful until it was learned that the metacercariae were infective only after sojourning at least eight days in the fish. Subsequently three cats, six rats, and one mink became infected from eating laboratory infected fish. The number of adult worms found in these animals was less than in those fed naturally infected fish. This is to be expected as the number of cysts in these small fish, even when they are heavily infected can not compare with the number in the larger naturally infected fish.

Although the metacercariae, eight days in the fish, are infective and develop to maturity in the cat, their cercarial organs are not entirely lost. In one case, a cat was fed fish which had been exposed eight days previously to cercariae. After three days the cat was autopsied. The maturing flukes still possessed their stylets, the average length of several stylets being 4.5 μ. Other flukes, also eight days in the fish which were allowed to mature in a cat were indistinguishable from the other mature flukes studied.

In the mammalian host the flukes are found in greatest abundance in the upper third of the small intestine, but in heavy infections may be distributed throughout the entire intestine. Sections of the infected intestine show the flukes imbedded in the mucosa. The worms can be found in the opened intestine only with the aid of the dissecting microscope or hand lens.

There is as yet no conclusive evidence as to the pathogenicity of this fluke. On the fur farm where the parasite was found in greatest abundance many dead mink were found with extremely heavy infections. In one case the number was estimated as ten thousand or more by counting the worms in measured samples of a suspension of the intestinal contents. Although it is hardly conceivable that such a multitude of parasites would not injure the host it is difficult to attribute the death of the animal to the parasites alone. There is a certain degree of inflammation of the intestine in the severe cases, but there is no enteritis comparable in extent or severity with that produced in dogs by *Nanophyetus salmincola*. (Simms, Donham, and Shaw, 1931, and Hoeppli, 1926.) Furthermore it was difficult, on the fur farms, to ascribe the death of a mink to its parasites in the absence of marked lesions or inflammation as unparasitized mink were also found dead from no obvious cause. An observation that has some bearing here is that fecal samples of 150 mink chosen at random showed a 22 per cent incidence of the fluke and 23 per cent of fifty-eight mink found dead were infected. If this parasite were a significant factor in the loss of the mink, one would expect to find a much higher percentage of infection in the dead than in the living animals.

The apparent close relationship of *Sellacotyle mustelae* to the salmon poisoning fluke of dogs, *Nanophyetus salmincola,* suggested that the former species might be pathogenic to dogs or some other animals even if not for the mink, the natural host. Donham (1925a, 1925b, 1928), Simms, Donham, and Shaw (1931), Simms, Donham, Shaw, and McCapes (1931), and Simms, McCapes, and Muth (1932) have shown that *Nanophyetus salmincola* causes an acute and often fatal disease in dogs. Seven days after being fed infective material, the animal develops a high temperature. During the next seven days the temperature drops below normal, the animal becomes very emaciated and dies, from fourteen to twenty days after infection. There is almost 100 per cent mortality but the few dogs that recover are immune. One hundred cysts are often enough to produce the disease.

In view of these facts an attempt was made to build up as heavy an infection as possible in one of the dogs. It was fed eight of the heavily infected bull heads from the Popple River, and although fluke eggs became abundant in the feces, there were no visible symptoms and the temperature remained normal. None of the other experimental animals showed symptoms attributable to the parasites.

X. DEVELOPMENT OF THE EGGS

The eggs of *Sellacotyle mustelae* are discharged in the unsegmented state, only yolk cells being visible inside the shell. In water they embryonate in thirty-five days, the motile miracidia being clearly visible through the egg shell. Hatching has not been observed and attempts to infect snails in the laboratory by placing eggs in the aquaria have been unsuccessful so far.

XI. DISTRIBUTION

During the years 1929 to 1931, while engaged in a general study of mink parasites, the author encountered *Sellacotyle mustelae* occasionally. It was found in 11 of 326 carcasses of mink from various fur farms near St. Paul. Eggs of this parasite were found in 26 of 358 random fecal samples of mink from the same farms. Although the history of these mink could not be determined, it is known that a large proportion of the farm mink during those years were originally wild. There is no evidence that the mink became infected on these farms. The fur farm which supplied much of the material for this study, differed from the majority in that local fish were fed to the mink and conditions were favorable for the development of *Sellacotyle*. Specimens of this fluke, collected from mink, have been received from R. G. Law, of the Ontario Government Experimental Fur Farm at Kirkfield, Ontario. According to Law (1930) this species (described as similar to *Nanophyetus salmincola*) is not uncommon in mink in that region.

Sellacotyle mustelae has been found commonly in the few wild mink examined. Below are listed the records of its collection in mink in Minnesota.

TABLE I.—*Collection Records of Mink Infected with Sellacotyle mustelae.*

Locality	Date	Accession No.
Rice Creek, Anoka County	2/22/31	80
Minneapolis	3/ 3/31	81
Deer River, Itasca County	1/26/32	21
Popple River, Itasca County	6/26/32	69
Sec. 25 of Twp. 150–27, Itasca County	7/ 6/32	73
Sec. 11 of Twp. 146–25, Itasca County	8/ 1/32	77

Metacercariae were found in the fish host in a number of localities in Itasca County, and a few other places in the state. In the following table are listed the collections of bullheads with a notation as to their infection.

It is seen that in each case when the parasites are present, they are found in all the fish examined.

Lightly infected fish were found in a number of large lakes (Round, Squaw, Monson, Cut Foot Sioux, and Mud Lakes).

TABLE 2.—*List of Collections of Sellacotyle mustelae in Ameiurus melas in Minnesota.*

Locality	Date	Fish infected	Fish uninfected
Popple River, Itasca County	7/ 2/32	34	0
Popple River, Itasca County	7/27/32	26	0
Bowstring River, Itasca County	7/ 8/32	25	0
Cut Foot Sioux Lake, Itasca County	7/12/32	32	0
Creek in Twp. 60–26, Itasca County	7/19/32	0	4
North Star Lake, Itasca County	7/19/32	0	2
Outlet of Johnson Lake, Twp. 59–26, Itasca County	7/19/32	0	3
Round Lake Twp. 148–58, Itasca County	7/22/32	7	0
Squaw Lake Twp. 148–27, Itasca County	7/23/32	20	0
Shallow Pond, Twp. 148–28, Itasca County	7/23/32	10	0
Blue Lake, Shakopee, Minn.	8/10/32	0	60
Monson Lake, Kandiyohi County	8/26/32	20	0
Mud Lake, Kandiyohi County	8/26/32	34	0

XIII. SUMMARY

Sellacotyle mustelae n. g., n. sp., a new trematode from the mink is described. It is a minute form inhabiting the small intestine of carnivores and although it has been collected only from the mink, it has been reared experimentally in the mink, dog, cat, rat, ferret, fox, raccoon, and skunk. No marked pathogenic effect has been noted in any of these hosts.

This parasite appears to be closely related to but not congeneric with the salmon poisoning fluke, *Nanophyetus salmincola* of the family Troglotremidae. For the two genera, *Nanophyetus* and *Sellacotyle* the subfamily *Nanophyetinae* is erected.

The cercariae develop in the snail, *Campeloma rufum*. They are microcercous, armed forms possessing a prominent mucous gland near the posterior end. They penetrate and encyst in a wide variety of fish, but the most favorable host appears to be *Ameiurus melas*.

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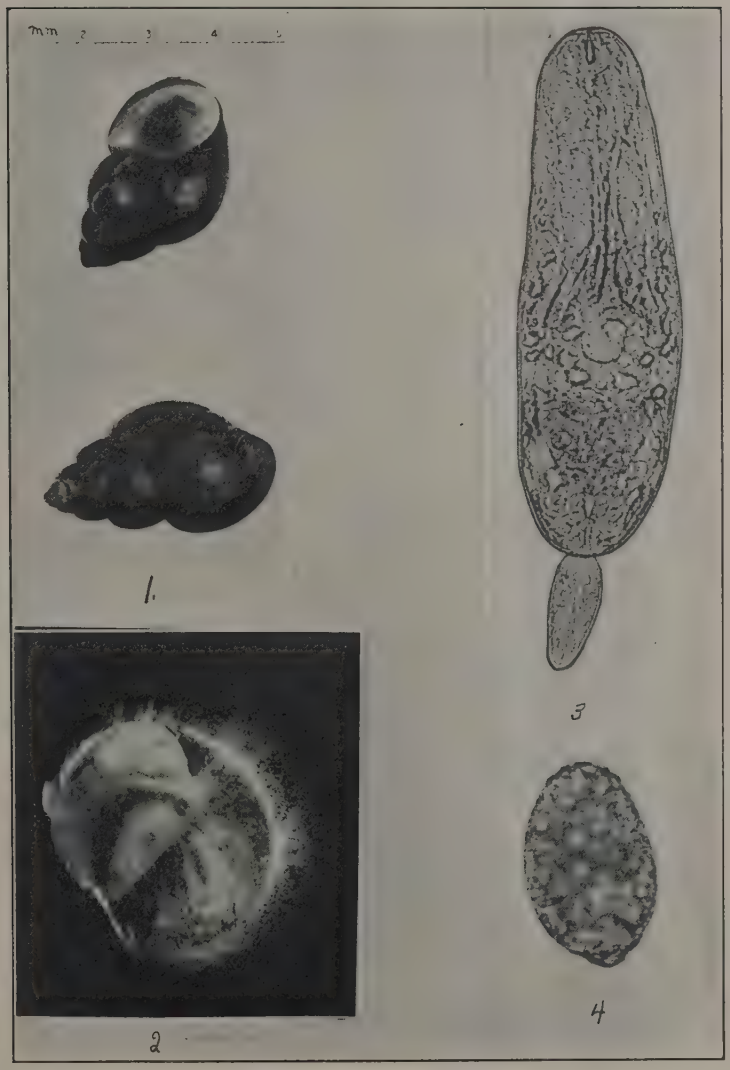
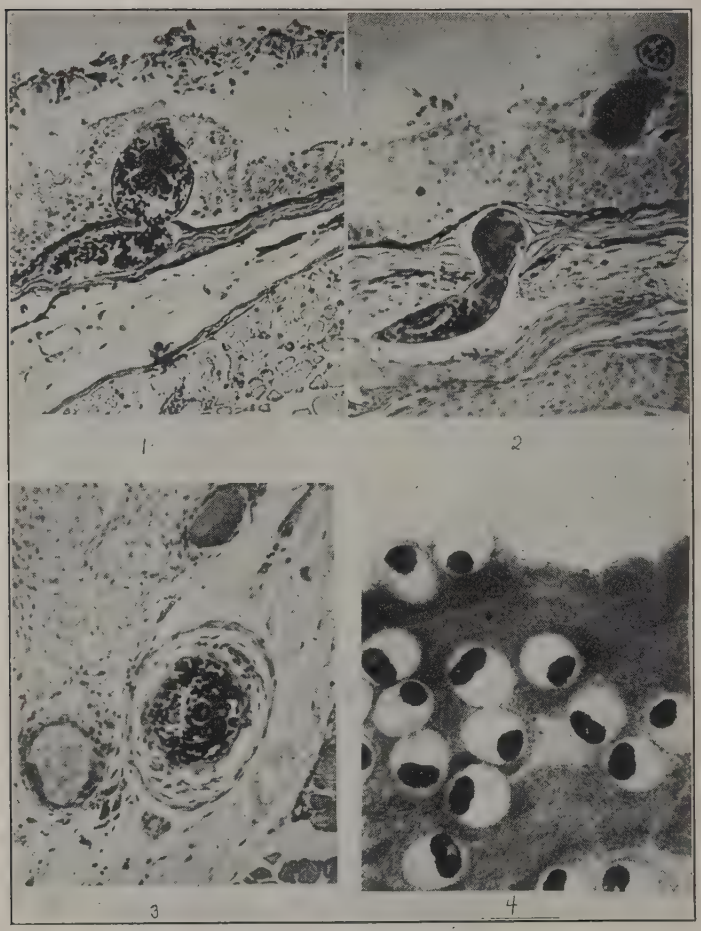
PLATE I



EXPLANATION OF PLATE I

Fig. 1. Shells of *Campeloma rufum*.
Fig. 2. Cercariae of *Sellacotyle mustelae* emerging from the snail host.
Fig. 3. Cercaria of *Sellacotyle mustelae*.
Fig. 4. Egg of *Sellacotyle mustelae*.



EXPLANATION OF PLATE II

Fig. 1. Section perpendicular to the epithelium of an experimental fish showing a penetrating cercaria. The anterior end of the cercaria has partly penetrated the dense subepithelial connective tissue layer and turned along it a short distance.

Fig. 2. Section perpendicular to the epithelium of an experimental fish showing a penetrating cercaria. Note the detached tail outside the epithelium of the fish.

Fig. 3. Section of a metacercaria in an experimental fish twenty-five days after infection. Note the fibrous capsule around the cyst.

Fig. 4. Fresh preparation of metacercariae in tissue from the base of the barbule of a naturally infected *Ameiurus melas*.

SOME OBSERVATIONS ON THE INTESTINAL PROTOZOA OF CALIFORNIAN LIZARDS

Wallace F. Wood

INTRODUCTION

With the exception of McFall's description of *Endolimax reynoldsi,* very little has been done on the intestinal protozoa of North American lizards. On the other hand, much has been published along this line in the Old World: Dobell, Wenyon, Prowazek, Grassi, Reichenow, Lavier, and others having described a number of species. The present work was commenced in the fall of 1928 to ascertain whether the intestinal protozoa of certain Californian lizards would prove of any special interest. To date a series of one hundred and twenty lizards representing sixteen species has been examined.

The study, however, was carried on chiefly with the various *Scelopori, Uta stansburiana,* and *Xantusia vigilis,* in which nine species of intestinal protozoa have been found. The blood was not studied extensively, although occasional films were made, and in one instance, an intracellular form of a haemogregarine was encountered in the blood of *Sauromalus obesus.*

The writer wishes to express his gratitude to Prof. John F. Kessel for the opportunity of working in his laboratories, as well as for his personal interest and many helpful suggestions.

METHODS

Saline smears direct from the intestine supplemented by occasional iodin-eosin and Janus Green B preparations, and slides fixed in warm Schaudinn's, and stained in Heidenhain's haemotoxylin were used throughout the work. Proper fixation of the flagellates with the exception of the larger trichomonads was generally difficult to obtain, as the faecal material usually consisted of chitinous insect fragments. Various devices including serum and albumin fixatives were tried without success. The most advantageous method was to use the Schaudinn's solution at 60° C., or even slightly higher. This procedure produced no evident artifacts.

Proteromonas lacertae (Grassi, 1879). (Figs. 1–4.)

This species was included by Grassi in his genus *Monocercomonas,* and was placed by Prowazek (1904) in the genus *Bodo.* In 1911 Alexeieff described another form from the intestine of Urodeles, and united the two species in the genus *Heteromita.* This organism has also been

widely known under the name *Prowazekella* proposed for these flagellates by Alexeieff. In the present study trophozoites of *P. lacertae* have been found in great numbers in the intestine and cloaca of seventy-five lizards of thirteen different species, *Dipsosaurus dorsalis, Sauromalus obesus, Callisaurus ventralis, Uma notata, Uta mearnsi, Uta stansburiana, Sceloporus occidentalis bi-seriatus, Sceloporus graciosus vandenburgianus, Sceloporus magister, Phrynosoma blainvillei, Xantusia vigilis, Lacerta muralis,* and *Lacerta viridis.* Cysts of the organism were found on four occasions only, twice in *Xantusia vigilis,* and once in each of the *Lacertas* examined. Large rounded forms, apparently pre-cystic in character, were encountered several times in *Sceloporus occidentalis biseriatus* and *S. magister.*

Chilomastix sp. (Figs. 5–6.)

So far as known to the writer, the only previous observer to publish an account of *Chilomastix* from the intestine of lizards was Wenyon (1921). He observed a small form in the intestinal tracts of *Lacerta agilis* and *Agama stellio* of Egypt, but gives no details and there is little to be derived from his figure. In this study flagellates of the genus *Chilomastix* have been found several times, once in *Callisaurus ventralis,* once in *Sauromalus obesus,* and on five occasions in *Sceloporus occidentalis.* Cysts were discovered only twice, five being seen in all. It is noteworthy that two of these were binucleate. The organism is very small, ranging from 4 μ to 8 μ in length. It presents no apparent morphological differences from the human form. By means of its size it may be distinguished from *C. caulleryi* Alexeieff of amphibians, which attains a length of 20 μ to 25 μ. It is interesting to note that Alexeieff concluded that *C. caulleryi* was morphologically identical with the human form. In order to determine the specific relationships of organisms belonging to such genera as *Chilomastix* and *Trichomonas,* thorough morphological studies together with suitable cultivation and cross infection experiments would prove of great interest.

Trichomonas lacertae Prowazek, 1904.

Prowazek (1904) described and figured quite poorly a trichomonad flagellate from the intestine of lizards of the genus *Lacerta.* Wenyon (1921) figured a small flagellate from the intestine of *Lacerta agilis* and *Agama stellio* as *Trichomonas lacerta.* Wenyon (1926) considers it probable that *T. mabuiae* described by Dobell (1910) from a skink of the genus *Mabuia* is identical with *T. lacertae.* No organism could be identified from Prowazek's and Wenyon's figures and descriptions. A few small trichomonads were encountered by the writer in the two *Lacertas* observed in the present study. Since these hosts were the same as

those from which Prowazek obtained his trichomonads, it seems logical to assume that the flagellates are *Trichomonas lacertae*. Similar organisms have been seen by the writer in all species of lizards studied except *Anniella pulchra* and *Gerrhonotus scinicauda*.

Trichomonas augusta Alexeieff, 1911. (Figs. 7–9.)

This trichomonad, which was found in all but one of a series of thirty-two specimens of *Xantusia vigilis*, but in no other lizard examined, was one of the most interesting forms observed. As there are several good detailed descriptions of *Trichomonas augusta* to be found in the literature, it would be superfluous to give such a description here. Many dividing individuals were seen. These correspond to the descriptions given by Kofoid and Swezy (1915). Chromosomes were distinct, five as stated by Kofoid and Swezy being the apparent number. It is interesting that no lizard but *Xantusia vigilis* has been found to harbor this organism. It would seem that *Uta stansburiana* and *Sceloporus magister*, which dwell in and around the fallen tree-yucca habitat of the *Xantusia*, would have a splendid opportunity to acquire this *Trichomonas*.

Eutrichomastix lacertae (Butschlii, 1844). (Fig. 10.)

The genus *Eutrichomastix* is very similar to *Trichomonas*, but differs from the latter in the absence of an undulating membrane. This similarity of the two genera has led to some confusion, supposed species of *Eutrichomastix* having been based on degenerate trichomonads. However, there seems little doubt but that two genera should be recognized. Many species of *Eutrichomastix* have been described from various reptilian hosts, but it is quite possible that all are the same form, *E. lacertae*. In this work these flagellates were found in the hindgut of *Uta stansburiana, Uta mearnsi, Sceloporus occidentalis bi-seriatus, Sceloporus graciosus vandenburgianus, Sceloporus magister*, and *Xantusia vigilis*. They seemed never to occur in large numbers, however.

Retortomonas melolonthae (Grassi, 1879). (Figs. 11–12.)

There is considerable doubt as to the correct name of this form. Wenyon (1926) discusses the question in some detail and reaches the conclusion that *Retortomonas* is the only generic name available. A number of these organisms have been found parasitized by a *Sphaerita* or a similar organism. Observed in haematoxylin-stained slides the flagellate seems filled with large black granules about 0.5 μ in diameter. These are usually arranged in one or two spherical clusters. *R. melolonthae* has been observed in various insects, and in the intestinal tract of amphibians by Swezy (1916). In the present study it has been found in lower gut of five specimens of *Sceloporus occidentalis bi-seriatus*, in

three of *Uta stansburiana,* and in three of *Xantusia vigilis.* In one *Xantusia* it was the only protozoan present and existed in the lower gut and cloaca in enormous numbers.

Entamoeba lacerticoli n. sp. (Figs. 13–16.)

An *Entamoeba,* probably seen but unnamed by Dobell (1914), Wenyon (1921), Franchini (1921), occurring in the lower intestine and cloaca of various lizards.

Diagnosis: Eccentric karyosome and large number of cytoplasmic inclusions distinguish this form from *E. varani* Lavier, 1928. The customary formation of eight-nucleate cysts, and the splinter-like chromatoidals differentiate it from the human *E. histolytica*; the hyaline pseudopodium and rapid streaming movement from *E. coli.*

Description: Trophozoites—20 μ to 45 μ in diameter; normally very active with a rapid streaming movement; a narrow margin of hyaline ectoplasm at the extreme anterior end of the motile organism; nucleus clearly visible in the living animal, and is a relatively large structure; karyosome eccentric, often lying close to the peripheral chromatin, which is regularly beaded on the nuclear membrane; many cytoplasmic inclusions, bacteria, flagellates, crystals and of undetermined nature.

Cysts—relatively uncommon; eight nucleate and very similar to those of *E. coli*; nuclear chromatin irregularly arranged in one to four masses on the nuclear membrane; karyosome small, eccentric; chromatoidal bodies splinter-shaped or thread-like, lacking in over half the cysts seen.

Occurrence: Observed by Dobell (1914) in *Lacerta muralis*; by Wenyon (1921) in *Lacerta agilis* and *Agama stellio* of Egypt. The writer has found it very common in *Xantusia vigilis,* and encountered it also in *Sceloporus occidentalis bi-seriatus, S. magister* and *Uta stansburiana,* and has found it less frequently in *Dipsosaurus dorsalis, Callisaurus ventralis, Sauromalus obesus,* and *Sceloporus graciosus vandenburgianus.*

This form is certainly different from Lavier's *Entamoeba varani* from *Varanus niloticus* of North Africa, but seems without much doubt to be that seen and figured by Dobell and Wenyon. It seems well to let it go no longer unnamed, and so the writer wishes to propose the name *Entamoeba lacerticoli* for this species.

Valkampfia reynoldsi (McFall, 1926). (Figs. 17–18.)

This organism was described from the intestine of *Sceloporus undulatus* as *Endolimax reynoldsi* by McFall in 1926. He described trophozoites, precystic forms and cysts in great detail. In this study an amoeba that agrees well with McFall's description has been seen on three occasions, twice in *Sceloporus occidentalis bi-seriatus,* and once in great number in *Uta mearnsi.* Trophozoites were found only in the ileum and encysted on passing into the rectum and cloaca. The writer concurs with Doflein (1928) in the opinion that this amoeba should be placed in the genus *Valkampfia* since only mononucleate cysts are formed.

Nyctotherus sp.

An organism of this genus was encountered several times in *Xantusia vigilis,* and on one occasion in the chuckwalla, *Sauromalus obesus.* This ciliate varied from 70 μ to over 100 μ in length, and was slightly more than half as broad. However, as the organism was no where plentiful, and as the preparations were not wholly satisfactory, the writer will not give a detailed description at the present time.

LEPTOMONAS-LIKE FLAGELLATE

In addition to the foregoing organisms, several Leptomomas-like flagellates were encountered in the hind gut of one specimen of *Xantusia vigilis.* As similar organisms were never encountered again, it seems best to give them only this brief mention.

CULTURE WORK

While no special attempt was made to cultivate the organisms found, all, with the single exception of *Nyctotherus,* have been maintained for varying lengths of time in L. E. S. medium, modified to contain one part of serum to twelve parts of Locke's solution. Temperature appeared to be the greatest factor in the success or failure of the cultures. The best growth was obtained at room temperatures, the Entamoeba being maintained twenty days. In a refrigerator the cultures also did fairly well; multiplication of the protozoa was slow but bacterial growth was at a minimum. In tubes at 37½ C. the bacteria soon dominated the cultures. The temperature seemed to influence successful growth by affecting the flora of the culture, rather than by acting *per se* upon the protozoa.

DISCUSSION

Among the interesting problems suggested by this study is the mode of transmission of the organisms. The only encysted forms found, and these rarely, were of the *Chilomastix,* the *Proteromonas* and the *Entamoeba.*

The lizards here studied may be divided into two groups as regard their habitats: 1. those which are desert dwellers of diurnal habits, or inhabit dry sunny places, and 2. those which are nocturnal and secretive in habit, or live in relatively moist localities.

The representatives of the first group, especially several of the desert species such as *Dipsosaurus, Sauromalus, Callisaurus,* which have shown heavy infections, are active chiefly during the warmer portions of the day in spring and summer. At this time the temperature ranges from 85° F. to 115° F., and the rocks are almost unbearable to the touch. Cysts and especially trophozoites perish very rapidly under such condi-

tions. In the feces of the chuckwalla, *Sauromalus obesus,* which is by far the largest of our desert lizards, most of the free flagellates are dead twenty minutes after the voiding of the feces. This was in captivity, under moderate temperatures, sheltered from the sun, in an atmosphere of relatively high humidity. None of these lizards with the possible exception of *Dipsosaurus* is at all gregarious.

It is possible that some of the flagellates such as *Retortomonas* may be insect forms taken in with the food, which set up a permanent infection in the reptile. *Retortomonas,* however, which seems the most likely organism to be transmitted in such a manner, has been found among lizards only in *Sceloporus occidentalis bi-seriatus.* Because of a more humid environment difficulty of transmission would probably not be so great in the case of these two.

Reichenow (1920) showed that at least in the case of *Eutrichomastix* an invasion of the blood stream might take place from the intestine. Mites which infest the reptiles become infected with the flagellates, and may retain the infection as long as thirteen days. Reichenow succeeded in infecting a newly hatched *Lacerta muralis* by feeding it upon these infected mites. The author has examined a number of ticks found upon various lizards with negative results.

It was also considered possible that transmission might occur at coitus. Juvenals are heavily infected, however. Under the apparent conditions it is puzzling to account for such heavy and universial infections among the desert dwelling lizards.

In the second group in which three lizards were studied, conditions seemed more favorable for easy transmission of the parasites. In those *Scelopori,* which inhabit timbered regions, where there is more moisture and shade, the infections were no heavier, however, than in those found in the exposed washes or on the desert. In the case of the nocturnal and secretive *Xantusia vigilis,* which is found only on the desert under the fallen limbs of tree yuccas (*Yucca arborescens* and *Y. mohavensis*), where there is comparatively a great amount of moisture, one would expect heavier infections. These lizards nearly all harbor three or four species of protozoa, and a number, five. In *Gerrhonotus,* also secretive in its habits, which frequents the more moist coastal regions the situation is different. Of the six negatives found in this series of 122 specimens, five were *Gerrhonotus* (the sixth was a single individual of *Anniella pulchra*). These five were the only individuals of the species examined. However, Professor R. M. Selle, of Occidental College, Los Angeles, has informed the author that he found an amoeba and flagellate in the intestine of an individual of *Gerrhonotus scinicauda.*

With reference to host-parasite relationships, it has been found that organisms morphologically identical have been found in widely varying

hosts. *Retortomonas,* which is probably of original insect origin, has been found in insect, amphibian, and reptile hosts. *Trichomonas augusta* occurs in both amphibians and reptiles. Of the nine species of protozoa observed, seven have been found in more than a single host. For example, identical *Proteromonas* have been encountered in nine hosts of five genera, representing three families, Iguanidae, Xantusiidae, Lacertidae. Certainly there is no rigid host-parasite relationship among these organisms.

SUMMARY AND CONCLUSIONS

1. The intestinal protozoa of 122 lizards of 16 different species have been studied.

2. Nine species of protozoa have been observed: *Proteromonas lacertae, Chilomastix* sp., *Trichomonas lacertae, T. augusta, Eutrichomastix lacertae, Retortomonas melolonthae, Entamoeba lacerticoli* n. sp., *Endolimax reynoldsi, Nyctotherus* sp.

3. Five specimens of *Gerrhonotus scinicauda* and one of *Anniella pulchra* were the only lizards found negative for protozoa.

4. *Xantusia vigilis* is more heavily infected than any other lizard examined.

5. The exact mode of transmission of the organisms is difficult to understand in view of the scarcity of encysted forms, the arid habitat and ungregarious life of most of the hosts.

6. Identical organisms were encountered in a variety of hosts.

7. The evidence obtained points toward the absence of a rigid host-parasite specificity within this group.

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Description of Plate

All drawings were made from specimens fixed in warm Schaudinn's solution and stained with iron haemotoxylin. All except No. 13 were drawn to an original magnification of 2200 and reduced one-third. No. 13 was drawn to a magnification of 4000 and reduced one-third.

Fig. 1. *Proteromonas lacertae,* large trophozoite from the intestine of *Xantusia vigilis.*

Fig. 2. *Proteromonas lacertae,* small trophozoite from the intestine of *Xantusia vigilis.*

Fig. 3. Large rounded trophozoite of *Proteromonas lacertae* from *Sceloporus magister.*

Fig. 4. Eight nucleate cyst of *Proteromonas lacertae* from *Xantusia vigilis.*

Fig. 5. *Chilomastix* sp., trophozoite from the intestine of *Uta stansburiana.*

Fig. 6. Binucleate cyst of *Chilomastix* sp. from *Sceloporus occidentalis.*

Fig. 7. Large trophozoite of *Trichomonas augusta* from *Xantusia vigilis.*

Fig. 8. Trophozoite of *Trichomonas augusta* from *Xantusia vigilis.*

Fig. 9. Dividing trophozoite of *Trichomonas augusta* from *Xantusia vigilis.*

Fig. 10. *Eutrichomastix lacertae* from *Sceloporus occidentalis.*

Fig. 11. *Retortomonas melolonthae* parasitized by a *Sphaerita* (?) from *Xantusia vigilis.*

Fig. 12. *Retortomonas melolonthae* from *Sceloporus occidentalis.*

Fig. 13. Cyst of *Entamoeba lacerticoli* from *Uta stansburiana.*

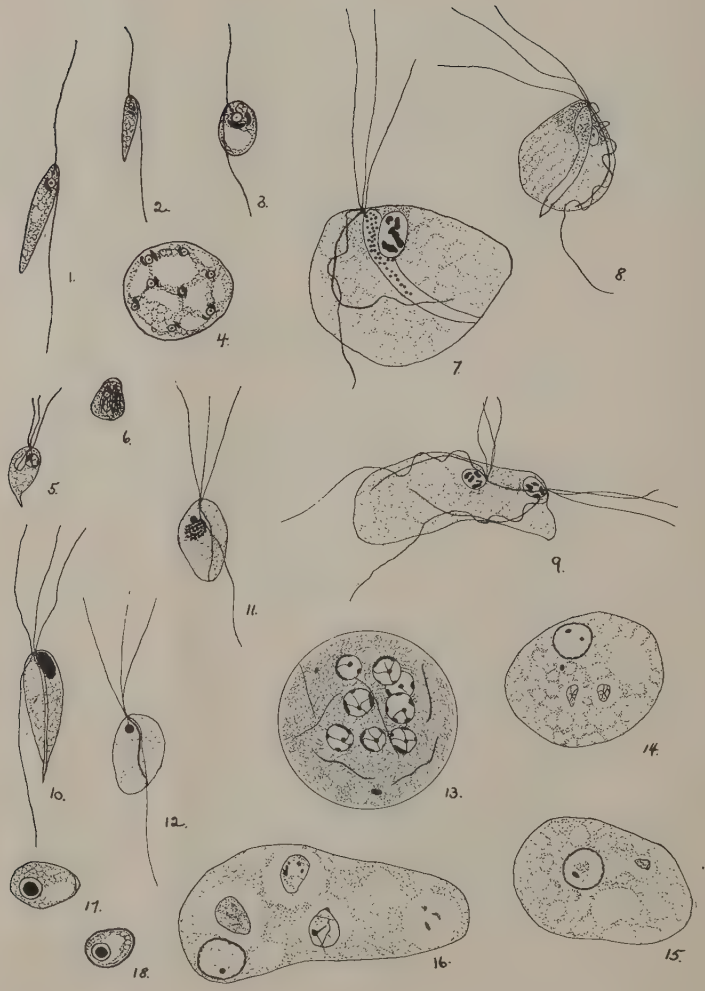
Fig. 14. Trophozoite of *Entamoeba lacerticoli* from *Uta stansburiana.*

Fig. 15. Trophozoite of *Entamoeba lacerticoli* from *Uta stansburiana.*

Fig. 16. Trophozoite of *Entamoeba lacerticoli* from *Xantusia vigilis.*

Fig. 17. Trophozoite of *Valkampfia reynoldsi* from *Uta mearnsi.*

Fig. 18. Cyst of *Valkampfia reynoldsi* from *Uta mearnsi.*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SEASONAL FLUCTUATION IN THE NUMBER OF EGGS OF TRICHOSTRONGYLID WORMS IN THE FAECES OF EWES

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An account recently appeared in this journal of some work carried out by Zawadowsky and Zvjaguintzev (1933) on the seasonal fluctuations in the number of eggs of *Nematodirus helvetianus* in the faeces of a llama. As the observations of these investigators compare very closely with some which have been made at this experimental station on the fluctuations in the numbers of eggs of trichostrongylid worms disseminated in the faeces of ewes, it seems to be worth while recording the data, which although of no great value in themselves, give confirmation to some of the observations of Zawadowsky and Zvjaguintzev but suggest an alternative interpretation of their results.

The inquiry into the amount of infective material disseminated by the ewes throughout the year was originally undertaken in order to obtain some idea of the relative importance of adult sheep as a reservoir of infection for the lambs. Ewes almost invariably carry a few trichostrongylid worms and outbreaks of disease with heavy losses amongst the adult sheep are by no means unknown. The possibility of their playing a part of major importance in producing heavy infection in the lambs, therefore, appeared to be worth a little investigation.

Twelve average ewes were chosen for this purpose from the experimental station flock and samples of their faeces collected on the same day of each of sixteen consecutive months. An average egg count was made by thoroughly mixing equal weights of faeces taken from each of the twelve samples and estimating the number of eggs per gram of the mixture by a standard dilution technique.

The laboratory flock grazes along with the cattle on 30 acres of good grass land. During the two seasons in which these observations were made, the lambs were born in February and March and for those two months the ewes were kept in the buildings and received hay and a little concentrated food but no grass or green food of any kind. For the remainder of the year they received no concentrated food at all but subsisted entirely upon the grass from the 30 acres of pasture.

The twelve ewes were infected with species of *Trichostrongylus, Ostertagia, Haemonchus, Cooperia,* and *Bunostomum,* and although the multiple infestation detracts in part from the value of the results obtained, the similarity in the fluctuations in the egg count with those

recorded for *Nematodirus* in the llama is sufficiently marked to suggest the operation of a common factor in both instances.

The results are shown on the accompanying graph, and represent a gradual rise from less than one hundred eggs per gram in November, December, January, and February to a peak in June, of seven hundred eggs per gram in 1932 and five hundred in 1933.[1]

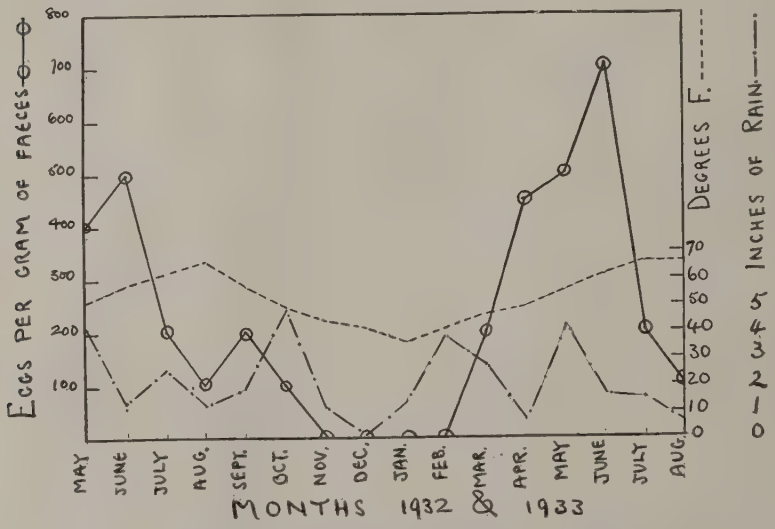

FIG. 1. Chart showing rise and fall in the numbers of eggs per gram of faeces disseminated by the twelve ewes. Also the mean monthly temperatures and the monthly rainfall throughout the same period.

Zawadowsky and Zvjaguintzev do not state the actual number of eggs per gram, but only relative numbers as obtained by Fulleborn's technique, so that the actual counts cannot be compared; but their figures show that the numbers of eggs remained very low from January to June, rose to a peak in August and fell again to a low count in January. It is thus seen that in both instances the number of eggs disseminated was low during the winter months, rose in the spring, and fell again in the autumn. Observations made by Turner and Moon (1928) on 38 lambs

[1] Since this paper was written an opportunity has been afforded for repeating the observations on another group of 12 ewes kept on the laboratory farm under conditions similar to those described for the previous group. The observations confirm those which are recorded in this paper, the composite monthly egg counts (eggs per gram of mixed faeces) between November, 1933, and October, 1934, being as follows: Nov., 200 e. p. g.; Dec., less than 50; Jan., 100; Feb., 100; Mar. 50; Apr., 150; May, 450; June, 500; July, 300; Aug., less than 50; Sept., 150; and Oct., less than 50.

at pasture showed a similar rise in the number of eggs which they disseminated; this reached a peak in August and declined again towards the approach of winter.

As an explanation of this rise and fall Zawadowsky and Zvjaguintzev suggest that the *Nematodirus* eggs which are disseminated throughout the winter months lie dormant during the cold weather and only give rise to infective larvae when the weather becomes warm enough for their development to take place, the infective material thereby being stored up for release in the spring; and they give an account of various observations to show that this actually does happen with the eggs and larvae of *Nematodirus*.

This explanation of the fluctuation in the numbers of eggs disseminated by infected animals does not, however, apply satisfactorily to the ewes. Winter temperatures are very moderate in the south of England, and it seems unlikely that any prolonged delay in the development of infective larvae could take place as a result of low temperatures. *Nematodirus* does not occur in the ewes at this experimental station and, as pointed out by Zawadowsky and Zvjaguintzev, eggs of the other species of trichostrongylids are less resistant; so that any accumulation of infection throughout the winter is unlikely to take place.

Although the most obvious explanation is that dry weather conditions in the latter part of the summer and low temperatures throughout the winter, being unfavorable to larval development result in a decrease in the intake of infective material, this should not be accepted too readily. The pasture concerned in these observations is grazed by a number of lambs as well as the ewes from July onwards, they acquire a fairly heavy infection, and disseminate 2000, 3000, or 4000 eggs per gram of faeces; an increase in the amount of infective material available on the pasture throughout the summer might therefore be supposed to take place, which, according to the theory of the Russian authors would show itself in an increase of eggs in the ewe's faeces. A second reason for doubting the "obvious" explanation is that the nutritious spring grass grows most rapidly in April, and happens to have been particularly luxuriant in the springs of 1932 and 1933, when these observations were made: this factor would tend to lower the intake of infective larvae, as the sheep would then be able to find sufficient grass on a smaller area and would therefore pick up fewer larvae than they would towards the end of the summer when grass is scarce and they are forced to eat the short herbage off a larger area of ground. Furthermore, the intake of larvae in the spring was, in this instance, quite definitely reduced during the period of eight weeks when the ewes were housed with the lambs, special precautions against infection having been observed during that period to ensure the rearing of worm-free lambs.

These observations are supported by those of Schmid (1933), who found the number of eggs of stomach worms disseminated by lambs to increase from May to September, but the number disseminated by ewes and yearlings, grazing the same pastures, to decrease throughout the same period; although, according to his account, the ewes appear to have been stall fed throughout the spring. These are circumstances which would have resulted in a low egg count in the ewes' faeces at the beginning and a rise towards the end of the grazing period, if it were true that the fluctuations were governed by intake of infective larvae.

The rise and fall in the number of eggs passed by the ewes does not appear, therefore, to synchronise with the rise and fall in the intake of infective larvae, and the operation of some other factor must be suspected.

Zawadowsky and Zvjaguintzev state "there can hardly be any doubt that the number of eggs is an index of the number of worms to be found in the intestine" and base their argument on this supposition; but the influence of immunity and diet of the host upon the egg production of parasitic worms is now well established, and the egg count can no longer be taken as a reliable indication of the numbers of worms present in an animal. Experiments, to be published elsewhere, have recently shown that the trichostrongylid worms present in lambs which have acquired an immunity to the ill-effects of parasitism produce only about one-twelfth the number of eggs produced by the same number of worms in susceptible lambs of the same age, and it is suggested that very possibly the infestation of the ewes remains at much the same level throughout the year, and that the fluctuations in the numbers of eggs which they disseminate is due to the influence of some factor upon the egg production of the parasites, rather than to the rate of intake of infective larvae.

The way in which this factor operates, or what it is that influences the parasites through the ewes, is not clear; the strain of giving birth to lambs and rearing them may play some part in the instance here reported, but presumably would not in the instance of the llamas, no mention of young llamas being made in Zawadowsky and Zvjaguintzev's paper, and it is not improbable that the explanation will ultimately be found to lie in differences in the composition of the herbage, and resulting differences in the nutrition of the animals at various seasons of the year.

SUMMARY

The number of eggs of trichostrongylid worms passed by breeding ewes is at a minimum during the winter months and rises to a peak in June, from which it falls again to the end of the year.

The rise and fall in the numbers of eggs passed by the ewes does not appear to synchronise with the rise and fall in the intake of infective larvae.

The explanation offered by Zawadowsky and Zvjaguintzev for a similar observation in the llama does not apply satisfactorily to this instance, and it is suggested that the fluctuations in egg counts are referable in both instances to variations in the rate of egg production of the adult worms rather than to fluctuations in the rate of intake of infective larvae.

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THE VALUE OF THE CASONI TEST IN DOGS

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For many years observers have noted the occasional occurrence of anaphylactoid skin manifestations following the rupture or puncture of a hydatid cyst. In 1911 Casoni (1) indicated that it was possible to utilize these reactions in the form of an intradermal test. Following his original publications many studies have been made on the Casoni reaction and its clinical value. Various changes in technic have been suggested for the test. Pontano (2), Serra (3), and Fairley (4) obtained high percentages of positive results with the test on patients with hydatid infections. Magath (5), Dew and Williams (6), and Dew, Kellaway and Williams (7) drew special attention to the significance of the primary wheal. Ithurrat (8) also called attention to the primary wheal and with the intradermal technic obtained 133 positive reactions out of 137 cases. Gasbarrini (9) utilized the appearance of the delayed reaction with local erythema and edematous infiltration as a positive indication of infection. Dew (10) has summarized the work on hydatid anaphylaxis in his monograph on Hydatid Disease.

During the past two years the authors have been investigating the possibilities of producing an artificial immunity against hydatid disease. Studies have been carried on both in the definitive and intermediate hosts. One of the major problems confronting the investigation has been the determination of the presence or absence of existing infection in the intestine of the definitive host (dog family) prior to immunization. Because of the known skin reaction obtained in the intermediate host with hydatid cysts it was felt that the possibility of positive skin reactions in the definitive host should be investigated with the hope that it might be of some aid in determining the existence of intestinal infection.

Sixty-one dogs of the non-descript types found in the streets of Beirut were used in the investigation. The study consisted of making preliminary Casoni tests in which both immediate and delayed reactions were read. The dogs were then sacrificed and thorough autopsies made in order to determine the presence or absence of intestinal infection with *Echinococcus granulosus*. All of the intradermal tests were made on the inner aspects of the thigh where the skin is hairless. Pooled, freshly prepared sheep hydatid fluid from fertile cysts obtained from the slaughter house was used for making the test. One-half cubic centi-

meter (0.5 cc.) of the fluid was injected intradermally in each animal. The immediate reactions were read at the end of fifteen minutes and again at the end of thirty minutes. Delayed reactions were read after twenty-four hours. Following the reading of the delayed reactions the animals were killed by injecting large doses of ether directly into the heart. The intestines were removed and labelled. Each intestine was cut open throughout its entire length and a thorough examination for *E. granulosus* made with the aid of magnifying lenses.

The results of these observations are recorded in table 1. Thirty-two (53.4 per cent) of the sixty-one animals gave immediate positive Casoni reactions. Five of the reactions were estimated as being equivalent to 4 plus, seven 3 plus, seven 2 plus and thirteen 1 plus positives.

TABLE 1.—*The Casoni test in dogs correlated with the presence of E. granulosus in the intestine.*

Immediate reaction	Delayed reaction		Autopsy findings—*E. granulosus*	
	Positive (1 cm. diam. or more)	Negative (less than 1 cm. diam.)	Present	Absent
4 plus	3	2	1	4
3 plus	7	0	3	4
2 plus	6	1	1	6
1 plus	8	5	3	10
Negative	12	17	8	21

Thirty-eight (62.3 per cent) of the animals gave positive delayed reactions, the size of the reaction at the end of twenty-four hours being noted in the table. Twenty-four of the thirty-eight positive delayed reactions were observed in the thirty-two animals that had given positive immediate reactions. Fourteen positive delayed reactions were noted in animals that had given negative immediate reactions.

Only sixteen (26.2 per cent) of the sixty-one animals examined were found to have *E. granulosus* in the intestines on autopsy. Eight of these sixteen positive intestines were noted in animals that gave immediate positive Casoni reactions and eight of them were in animals that gave negative immediate Casoni reactions.

It is worthy of note that only one of the animals (number 24a) that gave a 4 plus immediate reactions was found to have *E. granulosus* in the intestine. This animal had a negative delayed reaction. Dogs number 18 and 9a had very heavy intestinal infections with *E. granulosus* but they gave only 1 plus immediate skin reactions. Number 18 showed a delayed skin reaction while 9a had no delayed reaction at all. Six animals (numbers 5, 19, 23, 4a and 20a) showed definite intestinal infec-

tion but gave neither immediate or delayed positive reactions. Number 23 of this group had a very heavy infection with adult *E. granulosus*.

DISCUSSION

In view of these findings it is quite obvious that it is not possible to utilize the Casoni intradermal reaction in dogs as an index of intestinal infection with *E. granulosus*. The infection in the definitive host is restricted to the intestine without migration through other tissues and no general sensitivity of the skin sufficient to give a positive intradermal reaction is to be expected. Since the absorption of antigenic substances probably does not take place through an intact intestinal mucosa, it is possible that superficial attachment of the parasites fails to produce sufficient damage to the mucosa to allow sensitization. We have been at some loss to explain the appearance of strongly positive immediate and delayed skin reactions in animals showing no evidence of intestinal infection at all. It may be that these animals had been previously infected. However, the incidence of *E. granulosus* infection observed at autopsy in this group (26.2 per cent) corresponds closely to the incidence that has been observed by the authors in a large series of several hundred dogs examined during the past two years.

SUMMARY

(1) A study of immediate and delayed skin reactions to the intradermal Casoni test followed by autopsy and careful intestinal examination was made in a group of sixty-one dogs (definitive hosts) with the idea of trying to correlate a possible relation between the skin reactions and intestinal infection with *E. granulosus*.

(2) No direct correlation was observed between the presence of *E. granulosus* in the intestine of the dog and positive skin reactions to the intradermal Casoni test.

(3) The Casoni test as used in hydatid disease in the intermediate host is of no value as an index of intestinal infection with *E. granulosus* in the definitive host.

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THE MAINTENANCE OF A STRAIN OF *BALANTIDIUM* IN LABORATORY RATS

Floyd O. Atchley

INTRODUCTION

Since Noller (1926) reported balantidia from the caeca of rats living near a pigsty, several other workers have either discovered infected wild rats or have experimentally inoculated laboratory rats (Schumaker, 1930; Andrews, 1930; Obitz, 1931, and Nagahana, 1932). Apparently, natural infections are contracted from pigs, because it is in their vicinity that infected rats have been found.

Previous investigators have been unable to maintain "pure lines" of balantidia *in vitro*. A "pure line" *in vitro* was secured and then a supply of the organisms was maintained in the cecum of rats.

MATERIAL AND METHODS

With the aid of a micropipette similar to that devised by Rees (1931), single balantidia obtained from the chimpanzee were isolated and each placed in a culture tube. A Ringer's-serum (25–50 per cent) medium with the addition of a small amount of rice starch was satisfactory. Following laparotomy, 3 white rats were intracecally inoculated 8 days after isolation with one of the "pure lines" after the manner of Andrews (1930) and Schumaker (1930). For a period of one year, consecutive groups of 3 rats each were infected at intervals of usually 3 to 6 days. To separate balantidia from either chimpanzee or rat fecal material, an apparatus resembling that of Baermann (Cort and Payne, 1922) for isolating helminth larvae was evolved. This greatly facilitated the handling of the ciliates. A diet containing 85 per cent carbohydrates (Schumaker, 1930) was fed the rats before and after inoculation. Examinations of the cecal contents were usually made at autopsy, but were sometimes made by first injecting 1 or 2 cc. of Ringer's solution into the cecum and then withdrawing a small amount. Often, however, rats found negative by this method were positive when killed and autopsied.

RESULTS

A total of 38 attempts at isolating individual balantidia resulted in 8 of the culture tubes being positive from 1 to 3 days later. Fourteen trials made with fecal extract as the culture medium failed. The 8 successes occurred among 24 tubes of Ringer's-serum (25 per cent–50 per cent) medium. The "pure line" originally chosen has been maintained for more than 1 year at the time of writing.

TABLE 1.—*Examinations of rats following inoculation with balantidia*

Days after inoculation		Positive	Negative	Total	Per cent positive
Group	Mean				
3–6	5.1	47	2	49	95.9
7–13	9.7	28	2	30	93.3
14–30	19.8	11	6	17	64.7
31–60	46.8	10	11	21	47.6
61–upwards	99.6	8	16	24	33.3
Total		104	37	141	73.8

Table 1 summarizes the observations recorded for 10 months in connection with the duration of the infections in the 141 rats used. Inasmuch as the strain of infusorians was usually transferred to a new group of 3 rats every 3 to 6 days, the largest number of examinations falls in this class. However, various periods of time were allowed to elapse in some cases before examining in order to check the course of the infections. No cysts were ever observed in the cecal contents or in the fecal material of the rats, although they were frequently seen in chimpanzee feces.

When fixed, sectioned and stained, the cecum of a rat, which had retained its infection for approximately 4 months, revealed no tissue damage. Macroscopic lesions were not apparent in the ceca of any of the experimental animals.

SUMMARY AND CONCLUSIONS

1. Individual balantidia from the chimpanzee have been isolated and shown to multiply *in vitro*.

2. The use of laboratory rats is quite satisfactory for maintaining a strain of balantidia over a long period and such a method is superior to that of *in vitro* cultivation.

3. These balantidia did not encyst in the rat.

4. Lesions due to balantidia have not been observed in the ceca of infected rats.

5. A Baermann-like apparatus has been employed to separate balantidia from fecal material of the rat or the chimpanzee.

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STUDIES ON *CLINOSTOMUM*. II. THE MIRACIDIUM OF *C. MARGINATUM* (RUD.)

WANDA SANBORN HUNTER AND GEORGE W. HUNTER, III[1]

The free-swimming larva of *Clinostomum marginatum* (Rud.) is usually pyriform in shape, the greatest width occurring at the eyespots which lie between the anterior 1/4 and 1/3 of the body length.[2] Total length varies between 85 and 130 μ depending upon amount of contraction; width at eyespot region ranges between 30 and 65 μ.

After hatching from the egg, the miracidium swims about actively, apparently purposively seeking its host; it rotates slightly on its longitudinal axis. Progress is typically forward and the eyespots usually uppermost.

There are twenty-one epidermal plates (fig. 1), arranged in four transverse tiers as in *S. douthitti* (Cort) (Price, 1931). There are six plates in the first row; these are roughly triangular with their apices at the base of the anterior papilla, their posterior rounded edges terminating just anterior to the lateral papillae. The second row consists of 8 long, narrow, nearly rectangular but bluntly rounded plates extending into the posterior half of the body. The four plates in the third row are the largest, being over half as wide as long with sharply rounded corners. The three remaining plates form the fourth row; these are triangular with their apices surrounding the posterior tip. One is always dorsal and from this side the edges of the other two plates are visible. The cilia covering each plate range between 10 to 12 μ in length in different body regions; apparently inter-plate spaces are without cilia. When the miracidium is extended, there is a wide space between the second and third rows of epidermal plates. The relatively large, small-nucleated cells of the sub-epithelium lie immediately beneath the epidermal plates.

The protrusible anterior papilla assumes a variety of shapes depending upon the degree of extension. It frequently appears bulbous distally; and the stylet which it carries is attached to the wall of the bulb. When unextruded the stylet is sheathed within the papilla even when the latter itself is partially extended (fig. 2). The anterior papilla may be withdrawn into the anterior end of the apical gland, or "gut," and when extended it appears to be a part of this gland. This gland extends half the distance from the anterior tip of the miracidium to the eyespots. When

[1] A contribution from the University of Michigan Biological Station and from the Department of Biology, Wesleyan University.

[2] This description of the miracidium of *Clinostomum marginatum* is based entirely upon living material secured from adult parasites from experimentally infected great blue heron.

the anterior papilla is extended the length of the simple sac-like gland is markedly shortened. Since no lumen is present, the contents of the "gut" appear granular (resembling a secretion) and since the span of life of a miracidium is short (a gut being therefore unnecessary) we cannot believe this structure functions as an intestine. This leads us to the opinion that the structure is a part of the penetration apparatus. As Lynch (1933) concurs in this opinion we are adopting his proposed name of apical gland for this structure. Associated with the papilla are three pairs of penetration glands which extend nearly to the eyespots.

Many large-nucleated cells are scattered throughout the anterior 2/3 of the miracidium; these cells lie between the epidermal plates and internal organs. Since the nuclei of these cells take the same stain as the penetration glands and they are definitely limited in distribution, it is improbable that they constitute a part of the sub-epithelium. These may be analogous to the two rows of cells found in *S. douthitti* by Price (1931). In the latter species, however, the cells were in definite rows, while in *C. marginatum* they have no definite arrangement.

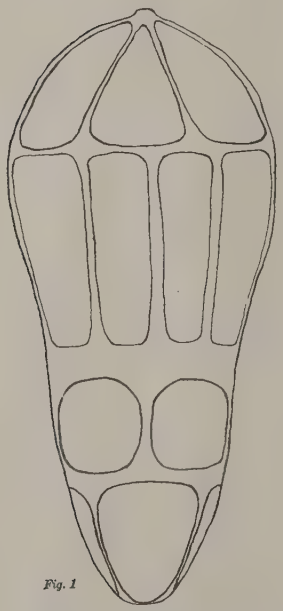
As in previously described miracidia (Price, 1931, Van Haitsma, 1931) a small papilla is found on each side of the miracidium between the first and second rows of plates. In *C. marginatum* this lateral papilla is composed of a single tube opening to the surface; it curves medianly and slightly posterior as it leads into a small vesicle near the anterior flame cells and vacuoles (fig. 2). The function of these organs is a moot question; that they have openings on the lateral surface is certain because the passing out of small drops of secretion (or excretion) from them has been observed by us. Cort (1919) and Faust and Meleney (1924) reported similar openings in other forms. No lateral processes were observed in any miracidia of *C. marginatum*.

Three prominent eyespots located on the mid-dorsal line just posterior to the lateral papillae are a distinguishing characteristic of *C. marginatum*. All three are cup-shaped, darkly pigmented and contain a lens. Two lie parallel with the third contiguous posteriorly forming a triangle. This third organ is smaller than the other two and is nearly spherical while the others are crescentic.

The large, bilobed brain appears as a granular mass; it is located immediately posterior to the eyespots. In some stages of contraction this brain mass appears very large, extending anterior to the eyespots. Typically, however, it extends from the eyespot region to the posterior edge of the second row of plates. Triangular masses of darker staining material were noted with both neutral red and brilliant cresyl blue stains at the postero-lateral angles of the brain.

Two pairs of flame cells are present; the anterior lie lateral to the brain between the eyespots and lateral papillae. The posterior pair are

found near the posterior edge of the third row of plates. The anterior and posterior flame cells on each side are connected by a straight tube which divides about midway and loops medianly and anteriorly almost to the eyespots before passing back, crossing under the main duct and looping around near the posterior flame cell; however, it is simply convoluted, soon leading to the excretory pore which lies on the lateral sur-



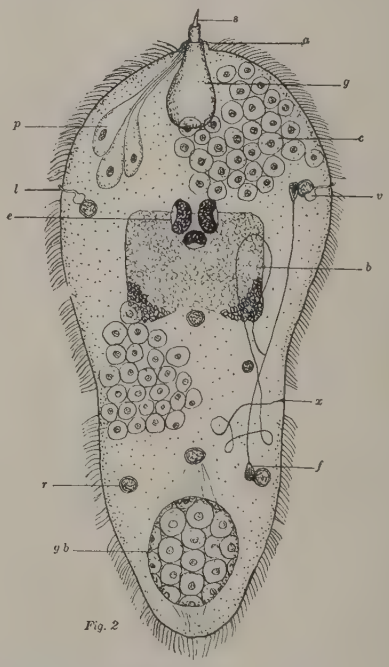


Fig. 2

Fig. 1.—Outline showing position and shape of epidermal plates. Fig. 2.—Schematic drawing of miracidium. *a*, anterior papilla; *b*, brain; *c*, large-nucleated cells; *e*, eyespots; *f*, flame cell; *g*, apical gland; *gb*, germ ball; *l*, lateral papilla; *p*, penetration glands; *r*, refractive vacuole; *s*, stylet; *v*, vesicle; *x*, excretory pore.

face between the second and third rows of epidermal plates. Although the position of the tubes, their convolutions and the flame cells is essentially constant they appear to shift with the contraction and extension of the miracidium. Pairs of large refractive vacuoles of constant number and location are found near the flame cells. Besides those near the protonephridial cells are 4 or 5 others as is shown in figure 2. These we believe function in some manner as part of the excretory system.

The germ ball, composed of large-nucleated cells varying in number from 9 to many, depending upon the stage of development, is held in a constant position in the posterior fourth of the body by fibrous strands analogous to connective tissue. Its shape varies only slightly, being typically spherical except during extreme extension of the larva, when it becomes somewhat elongate.

Yamaguti (1933) briefly describes a miracidium which he designates as *C. complanatum*. No excretory system, epidermal plates or penetration glands are described, which makes comparison with our form difficult.

Price (1931) suggests that the arrangement of the epidermal plates in the miracidia of *Strigea tarda* (Mathias, 1925), *Diplostomum flexicaudum* (Van Haitsma, 1931), and *S. douthitti* is another fact in support of the claim for relationship between the Strigeidae and the Schistosomatidae. If these plates indicate relationships, those described for *C. marginatum* would tend to place the Clinostomidae in the same order, Strigeatoidea.

Cort (1917) shows how the similarity of the excretory systems of cercariae demonstrate family relationships. Since this system is important in determining cercarial relationships, it is probably also worthy of consideration in the study of miracidia. The fact that the miracidium of *C. marginatum* resembles those of *S. douthitti* and of *D. flexicaudum* (Van Haitsma, 1931) in having two pairs of flame cells again suggests a relationship to the Strigeatoidea.

A study of the development of the excretory system through the cercarial to the adult stages of *C. marginatum* will doubtless add interesting data as to the relationships and ultimate classification of the Clinostomidae.

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THE PARTIAL PURIFICATION OF *BALANTIDIUM COLI* FROM SWINE[1]

R. W. Glaser and N. A. Coria

Barret and Yarbrough in 1921 (1) first cultivated *Balantidium coli* from man in a special medium. To keep the strains under cultivation it was necessary to subculture every second day and, even then, all of the protozoa died in some of the transplants and the procedure had to be re-repeated. Thus eleven successive transplants were made covering a period of 32 days. The frequent transfers at short intervals prevented the excessive growth of bacteria which Barret and Yarbrough felt was detrimental to the growth of *Balantidium.*

Rees in 1927 (2) modified the method of Barret and Yarbrough and applied it to the cultivation of the *Balantidium* of swine and of the guinea pig. He maintained his cultures at 36° C. for over fifty-four days, transplants usually being made at three-day intervals.

Scott in 1927 (3) cultivated *Balantidium coli* from the guinea pig. By frequent transfers to fresh tubes, the protozoa were kept alive for from three weeks to one month. Jameson in 1927 (4) studied the parasite from swine and strains of the ciliate were readily propagated for any desired period if subcultures were made every three to five days.

Schumaker in 1931 (5) showed that certain types of bacteria had an influence on the degree of infection in the pig with *Balantidium.* From his cultural work, he also came to the conclusion that bacteria contribute little, if at all, to the maintenance of the ciliate. This worker was also of the opinion that a difference may exist in the cultivability of separate strains of *Balantidium.*

From the publications reviewed, it is evident that *Balantidium coli,* no matter whether it is derived from the human host, from swine or from the guinea pig, is exceedingly difficult to maintain in cultures for any length of time. The suggestions made indicate that certain bacteria and their products exert a detrimental influence and that transplantation to fresh media at very short intervals is the only available means for keeping the parasite alive.

The present paper concerns itself primarily with results obtained by the elimination of many bacteria ordinarily present in cultures of *Balantidium.*

MEDIA USED

After trying various substances in different proportions, two simple media were adopted and used throughout the present work.

[1] From the Rockefeller Institute for Medical Research, Department of Animal and Plant Pathology, Princeton, New Jersey.

Semisolid medium.—250 cc. of sterile Ringer's solution, prepared according to the formula given by Schumaker in 1931, is warmed to 50° C. To this are added 25 cc. of 2 per cent standard nutrient agar, 1 gm. of sterile rice starch and 12½ cc. of sterile horse serum, not over two weeks old. The whole is mixed well, the pH adjusted to 7.2 to 7.4 and, under sterile conditions, 15 cc. amounts are poured into individual tubes which are placed in the refrigerator to congeal the medium. The medium is always warmed to 37° C. before using.

Liquid medium.—Each tube of this medium contains 9 cc. of the above Ringer's solution and ½ cc. of sterile horse serum to which is added a sprinkle of sterile rice starch.

PURIFICATION PROCEDURES AND RESULTS

In the following outline we have applied, in a modified form, certain technical procedures learned during the course of our former work with other protozoa (6).

The ilium and caecum of each pig was emptied and the mucosa, near the ilio-caecal valve, flooded with Ringer's solution warmed to 37° C. The mucosa was then scraped around the numerous crypts that occur in this region and about 2 cc. amounts transferred to the surfaces of one arm of "V" tubes,[2] each containing 15 cc. of the semisolid medium. The "V" tubes were incubated at 37° C. and complete migration downwards of the Balantidia occurred within 24 to 48 hours. By following this technique, 29 out of 40 cases, or approximately 73 per cent, in one set of samples, became positive. In our experience more positive initial cultures were obtained on the semisolid medium than was the case with the liquid media customarily used. Since movement was considerably restricted in the semisolid gel, bacteria did not sink or swim so rapidly as in a fluid. The protozoa, however, seemed to possess the necessary energy to overcome the gel obstacle and by migrating to the bottom of the tubes escaped many of the harmful bacteria. Initial cultures in liquid media became heavily contaminated much earlier. If cultures were established in ordinary tubes containing the semisolid gel it was impossible to make subcultures without again heavily contaminating these. For this reason the "V" tubes proved superior, because after the migration one could penetrate to the bottom through the other arm which remained sterile.

After the migration downward in the "V" tubes the Balantidia were removed through the sterile arm and again placed on the surface of the semisolid medium in one arm of a fresh "V" tube. This tube was incubated, and frequently examined with a hand lens during the process of migration. After the migration down in 24 to 48 hours the protozoa

[2] A "V" tube was described and figured in 1930 (6).

were again recovered. The process was always repeated five times, after which the ciliates were transferred to ordinary tubes of the semisolid gel. The final cultures after incubation ordinarily showed little clouding and did not smell badly.

The advantages of this technique were checked in another way. Fifteen samples obtained from the entrails of one pig were treated as described and fifteen more samples from the same pig were cultured by using the customary direct method and liquid media. The first set of samples yielded three tubes that were positive for *Balantidium,* whereas in the second set all of the tubes were negative and had a bad odor.

After the migration treatment, two cultures of Balantidia derived from separate sources were studied bacteriologically. Each strain was found contaminated with only two varieties of *Bacillus coli.* One *Balantidium* culture yielded *Bacillus coli communior* and *anaerogenes.* The other culture yielded the latter variety and *Bacillus coli communis.* All of our efforts to completely eliminate bacteria have so far proven unsuccessful, probably because the positive, geotropic response of *Balantidium* is exceedingly slow in contradistinction to that of many other protozoa.

Following the partial purification, *Balantidium coli* may be safely held in the semisolid gel for seven days without transplantation, indeed some strains remained active and transferable for from 10 to 12 days. One culture has been so carried for three months, another for over six months and a third for over two years.

ADAPTATION TO THE LIQUID MEDIUM

If transfers to the liquid medium were made after the initial five migrations in the "V" tubes, the Balantidia often did not develop. If, however, the partially purified cultures were carried for a period on the semisolid medium, a transition to the liquid environment was more easily effected. When this occurred the cultures could be maintained without frequent transfers.

At each transfer made once a week from the semisolid to the same medium, three transfers were also made to the liquid medium. One *Balantidium* strain did not adapt itself to this medium until the 5th transfer. At this transfer two out of three tubes containing the liquid became positive in 48 hours. One of three cultures of a second strain adapted itself to the fluid after the 6th passage in the gel. A third strain did not become adapted to the liquid until the 15th passage in the semisolid environment when two out of three cultures yielded a luxuriant growth between the 6th and 8th day. After adaptation to the fluid, it was possible to transfer the strains back to the gel and obtain successful cultures.

Balantidium cultures were often initiated in liquid media, but a failure to develop did not imply that a particular pig was negative for the

protozoa. Strains apparently varied in their cultivability in liquid media, a fact also noticed by Schumaker in 1931. However, the migration method in the semisolid gel proved much more reliable in this respect. The transition from the host to the gel did not appear so severe as was the case from host to the fluid environment. Slow adaptation to a liquid medium was obtained with most of the strains and when so adapted they developed for a long time without the necessity of the usual frequent transfers. As a matter of routine, most of the strains adapted to the fluid were transplanted at eight-day intervals, but the old cultures remained alive and transferable for two or three weeks more. Three strains remained viable and apparently normal without transplantation for 20, 28 and 33 days, respectively. The first of these has been transferred every 20 days and under these conditions has thrived for over 2½ years. Cysts have never been observed in any of our cultures.

SUMMARY

A semisolid and a liquid medium were used for the cultivation of *Balantidium coli* of swine. A partial purification of the ciliate was effected by permitting it to migrate through the semisolid gel in "V" tubes. By taking advantage of the described technique more positive initial cultures were obtained than by the usual procedures. This was due to the fact that many bacteria injurious to the protozoan were eluded. Following the partial purification, *Balantidium* was regularly held in the semisolid medium without transplantation for seven days and sometimes for from 10 to 12 days. *Balantidium* transferred from the purification tubes to the liquid medium often failed to develop. A slow adaptation from the semisolid gel to the fluid was obtained with most of the strains. When once adapted to the liquid environment certain strains of *Balantidium* remained alive and transferable for long periods of time. One strain has been transplanted every twenty days and has thrived for over two and one-half years.

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A NEW TREMATODE OF THE FAMILY GORGODERIDAE

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In a study of trematodes of the family Gorgoderidae a heretofore undescribed species was collected from the urinary bladder of the green sunfish, *Apomotis cyanellus*. During the summer of 1931 seven sunfish were examined and two were found to be infested with a total of ten specimens of the species in question. I propose to name this species *Catoptroides lohrenzi,* in honor of Dr. H. W. Lohrenz, formerly president and professor of biology of Tabor College, Hillsboro, Kansas.

Catoptroides lohrenzi n. sp. (Fig. 1.)

Specific diagnosis.—Catoptroides: Live trematodes sluggish in movement, opaque and of a dull, reddish-brown color with ovary and testes conspicuous in contrast as clear transparent structures; vitelline glands dark bodies visible in extended worm. Fully extended live specimens 6 mm. long by 3 mm. wide, while sexually mature specimens fixed in corrosive sublimate only 2.64 mm. in length, which is less than one-half the length of an extended living worm; pre-acetabular width 0.88 mm.; post-acetabular width 1.77 mm.; thickness of body 0.5 mm. Margin of flattened body smooth except for two or three deep notches on each side.

Fig. 1. *Catoptroides lohrenzi* n. sp., ventral view.

Pre-acetabular portion narrow and sharply set off from the broader caudal portion. Oral sucker terminal, 326 μ to 454 μ in diameter. Acetabulum slightly larger than oral sucker, nearly circular, 340 μ by 383 μ to 483 μ by 497 μ in diameter, and from 0.45 to 1.2 mm. from oral end. Pharynx absent. Oesophagus slender and variable in length. Intestinal rami first turn laterad and at level of anterior testis turn slightly mesad, thus making room for the massive reproductive organs, running slightly beyond the posterior testis. Testes large, nearly tandem in position, with four to eight very deep lobes in addition to several shallow notches; they occupy major inter-intestinal space and range from 256 μ by 340 μ to 525 μ by 667 μ in size, anterior one always smaller. Genital pore median, midway between junction of intestinal cruri and acetabulum. Cirrus apparently absent; prostate well developed.

Ovary slightly lobed, 185 μ to 255 μ wide by 312 μ to 398 μ long, placed laterally and in same level with anterior testis and may alternate with it, being either on left or right side. Vitellaria paired, oblong, between acetabulum and ovary and anterior testis; they are from 92 μ to 156 μ wide by 156 μ to 312 μ long, separated from each other by a distance equal to their own length. Mehlis's gland and ootype situated in median plane between vitellaria immediately behind ventral sucker. Laurer's canal not observed. Uterine coils very extensive, covering entire body region caudal to acetabulum save for portions occupied by other reproductive organs. Metraterm well developed. Eggs uniform in size, 27 μ by 34 μ.

Type specimen.—U. S. Nat. Mus. Helm. Coll. No. 8666.

Host.—Apomotis cyanellus Raf., the green or blue-spotted sunfish.

Location.—Urinary bladder.

Locality.—Cottonwood River, Marion, Kansas.

This species differs from the other known members of the genus in color and shape of the living worm, in being the smallest one in size when fixed, in having a large oral sucker, and in that the ratio between oral sucker and acetabulum is less than in most other species (1 : 1.1). The testes are large and very deeply lobed, being most like those of *C. lacustri* in this respect. Vitellaria are less lobed and the uterus is more extensive in distribution; the eggs are nearly as long as those of *C. lacustri,* but wider; only *C. macrocotyle* has larger eggs; in other species of the genus the eggs are smaller than in *C. lohrenzi.*

In a review of the genus *Catoptroides* the writer (Loewen, 1929) overlooked a paper by MacCallum, 1917, in which *C. magnum* and *C. aluterae* are described. MacCallum in the same paper also refers *Xystretrum solidum* Linton, 1910, and *X. papillosum* Linton, 1910, to the genus *Catoptroides.* Travassos, 1920, created the genus *Macia* to include the two species described by MacCallum, with *C. magnum* as type. Linton's species are characterized by possessing a thickened muscular disc on the ventral side of the body, by the absence of an oesophagus, by the presence of a cirrus, and by a highly anterior position of the genital pore; these characters are of generic value and hence *X. solidum* and *X. papillosum* should not be retained in the genus *Catoptroides.* This leaves the following (six) species to be recognized as belonging to this genus: *C. spatula* (Odhner, 1902), type, *C. spatulaeforme* (Odhner, 1902), *C. macrocotyle* Lühe, 1909, *C. staffordi* (Pearse, 1924), *C. lacustri* Loewen, 1929, and *C. lohrenzi* Loewen (present paper).

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ZEUGORCHIS NATRICIS N. SP. A TREMATODE FROM THE WATER SNAKE

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Thirty specimens of an apparently new trematode for which the name *Zeugorchis natricis* is proposed were found in the uterus of a water snake, *Natrix sipedon* (L) collected from Wolf Creek, Grove City, Pennsylvania. The infestation occurred in each of the uteri for an area of about two centimeters beginning about six centimeters from the cloaca. The uteri were distended and the trematodes could be clearly seen through the uterine walls.

This trematode is placed in the genus *Zeugorchis* established by Stafford (1905) for the type species, *Z. aequatus,* a parasite from the esophagus and stomach of the common garter snake, *Thamnophis sirtalis* (L).

Zeugorchis natricis n. sp.

Family Plagiorchidae Luhe 1901; *subfamily* Reniferinae Pratt 1903

Zeugorchis natricis is an elongate worm with an average length of 6.34 mm. and width of 1.67 mm., the type specimen being 6.18 mm. by 1.48 mm. Skin entirely covered with stout spines. Oral sucker subterminal in position, measuring 0.631 by 0.554 mm. Acetabulum 0.569 by 0.539 mm., anterior to middle of body.

Prepharynx, 0.092 mm. long; followed by the pharynx, 0.2695 mm. in length. Esophagus, 0.231 mm. long, dividing into cura which extend to posterior level of testes.

Ovary, 0.379 by 0.364 mm., near right lower margin of acetabulum and close to dorsal body wall. Mehlis' gland posterior to ovary and receives transverse vitelline ducts which can be clearly seen in worms from which eggs have been extruded. Vitellaria composed of small follicles lateral to intestinal cura and extending from bifurcation to near end of cura. Testes in posterior region of middle third of body opposite one another. Vasa efferentia extend from anterior margin of testes to posterior region of acetabulum where they join and enter the large cirrus sac. Uterus tube coils caudad to near posterior end where it turns and passes forward between testes. At about middle level of acetabulum uterus continues as a muscular metraterm. Metraterm separated from cirrus sac and extends to genital pore which is median and in region of intestinal bifurcation.

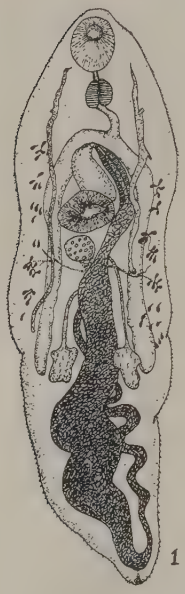
Excretory system Y-shaped and on each side of body, in anterior region, can be seen dark pigmented excretory tubes, extending from the region of oral sucker to about testes.

Type specimen.—United States National Museum Helminthological Collection No. 39503.

In addition to the type species the following have been reported from North American snakes: *Z. boscii* (Cobbold, 1859); *Z.* (=*Renifer*) *ancistrodontis* MacCallum, 1921); *Z. syntomentera* (Sumwalt, 1926); and *Z. megametricus* (Talbot, 1934). The following key will serve to distinguish the species of the genus.

Species of the Genus Zeugorchis

1 (2) Caeca extending to posterior end of body ..... *Z. boscii*
2 (1) Caeca not extending to posterior end of body 3



Zeugorchis natricis n. sp.

Fig. 1. Mature worm. ×16.
Fig. 2. Section through genital pore. ×37.
Fig. 3. Section through Mehlis' gland and vitelline ducts. ×37.

3 (4) Caeca not extending beyond acetabulum ..... *Z. megametricus*
4 (3) Caeca extending beyond acetabulum to region of testes 5
5 6) Caeca extending almost to posterior limit of testes ..... *Z. natricis*
6 (5) Caeca terminating between acetabulum and testes 7
7 (8) Worm about 7 mm. long; esophagus longer than pharynx. *Z. ancistrodontis*
8 (7) Worm 1.2–2.8 mm. long 9

9 (10) Oral sucker larger than acetabulum; esophagus longer than pharynx. *Z. aequatus*

10 (9) Oral sucker smaller than acetabulum; esophagus equal to pharynx. *Z. syntomentera*

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COMPARATIVE RESISTANCE OF BRONZE TURKEYS AND WHITE LEGHORN CHICKENS TO THE INTESTINAL NEMATODE, *ASCARIDIA LINEATA* (SCHNEIDER)[1]

J. E. Ackert and L. L. Eisenbrandt

In 1921 Ransom found that chickens were more resistant to the viability and growth of the gapeworm, *Syngamus trachea,* than were turkeys. His examinations of the tracheas of 635 chickens killed in Center Market, Washington, D. C., yielded no gapeworms. During the same period the tracheas of 386 turkeys were examined and in the following year 293, bringing the total turkey tracheas examined by Ransom to 679. The results of these examinations showed that 22.5 per cent of the turkey tracheas were infested with gapeworms.

As the chickens examined were adults or approximately full grown, Ransom carried out feeding experiments, giving embryonated eggs of gapeworms taken from turkeys to chickens of various ages with results as follows: chickens one to four weeks of age, 87.7 per cent infested; those five to eight weeks, 84 per cent infested; those nine to 20 weeks, 66 per cent infested; and chickens 21 weeks to adults, 29 per cent infested.

After these tests Ransom made others, the results from which indicated that as the chickens approached maturity they became very resistant to the gapeworms. For example, six adult chickens were fed embryonated eggs of the gapeworm. One chicken killed in 7 days was negative; a second chicken killed 8 days after feeding carried lung and trachea infestations of immature worms, the females in the trachea measuring only from 3 to 6 mm. in length. A third chicken killed 11 days after feeding was negative. Twenty-nine days after feeding a fourth was free from parasites. At this time the two remaining chickens were again fed gapeworm eggs. Eight days after this feeding the chickens were killed. One was free from parasites; the other had one worm in the lungs and a pair in the trachea. Thus while it was possible to infest adult chickens with gapeworms in some cases, the results showed that such chickens were very resistant to *Syngamus trachea.*

To compare the resistance of turkeys experimentally, Ransom fed gapeworm eggs to three turkeys nearly a year old. Nineteen days later one of the turkeys which was observed to be sick was killed. Its trachea contained 483 pairs of gapeworms, the female worms averaging 24 mm.

[1] Contribution No. 155 from the Department of Zoology, Agricultural Experiment Station, Kansas State College of Agriculture and Applied Science, Manhattan.

in length. The second turkey killed 81 days after feeding was infested with 22 pairs of gapeworms, the females averaging 33 mm. in length. The third turkey kept 10 months after feeding finally showed no gapeworm eggs in the feces. The results of the studies indicated that young chickens and adult turkeys manifested little resistance to gapeworms whereas adult chickens were markedly resistant to the viability and growth of the worms. These and other considerations led Ransom to think that the turkey is the natural host of the gapeworm, *Syngamus trachea.*

In 1929 Sandground after reviewing Ransom's gapeworm work[2] and that of Herrick (1926), who found evidence of marked resistance in growing chickens to the large intestinal roundworm, postulated the theory that the chicken is an abnormal host for this roundworm, *Ascaridia lineata* (Schneider). Sandground stated that abnormality of host must necessarily be an important if not a critical factor in any explanation of age resistance, the inference being that the turkey, from which this nematode was early reported, is the normal or natural host, and hence, less resistant to these nematodes. The phenomenon of age resistance is discussed by Ackert, Porter and Beach (1935).

Two opportunities to compare the resistance of turkeys and chickens to this intestinal nematode (*A. lineata*) have been afforded. The results obtained are here recorded.

MATERIALS AND METHODS

The turkeys[3] used in these experiments were secured as month old poults reared in clean quarters at the college poultry farm. The four ducklings used in the first experiment were likewise raised in clean quarters and secured at the college poultry farm. All chickens used in the experiments were secured as day-old chicks from accredited, commercial hatcheries and reared helminth-free in confinement until parasitized. At the beginning of each experiment all of the birds were assembled in adjacent pens and given the following ration, the turkeys and chickens being hopper fed and the ducklings pan fed: yellow corn meal, 45 gm.; cracked wheat, 15 gm.; oatona (steel cut oats), 17 gm.; alfalfa leaf meal, 4 gm.; meat meal, 10.5 gm.; powdered milk, 6.5 gm.; and cod liver oil, 2 gm.

[2] Attention is called to an inadvertent misquotation by Sandground (1929, p. 239) of Ransom (1921, p. 7). Sandground states, "While turkeys of all ages may harbor the parasites (*Syngamus trachea*) in large numbers without displaying any outward discomfort . . ."; whereas, Ransom states that after parasitizing three adult turkeys with *Syngamus trachea,* one of the turkeys observed to be sick 19 days later was killed. In its trachea were found 483 pairs of *Syngamus trachea.*

[3] Indebtedness is acknowledged to Mr. H. M. Scott, of the Department of Poultry Husbandry, for assistance in securing the turkeys and ducklings.

The worm eggs were secured from live *A. lineata* collected from chickens. The eggs were incubated in distilled water containing a trace of 2 per cent formalin at about 30° C. Parasitizing was accomplished by counting out the same number of embryonated eggs on a microscopic slide, wiping the eggs onto a bit of filter paper and inserting the filter paper into the esophagus of the fowl. At the close of the experiment the birds were killed, the contents of the intestines promptly flushed out, and the worms counted and measured (Ackert, Porter, and Beach, 1935). The criteria for comparing the resistance were the numbers and lengths of *A. lineata* removed from the groups of birds under comparison.

EXPERIMENTAL DATA

Experiment I. Four turkeys, four white leghorn chickens and four ducklings approximately six weeks old were each parasitized by being fed 100 embryonated eggs of the intestinal nematode, *A. lineata.* One month later the birds were killed and examined. Only three worms were found. One turkey harbored an adult female *A. lineata* and one chicken had two adult *A. lineata,* a male and a female. The ducks were all negative. While the number of worms was very small there was no evidence that the turkeys were less resistant to these worms than were the chickens.

Experiment II. Fifteen bronze poults (turkeys)[4] one month of age and 25 single comb white leghorn chickens of the same age were used in this experiment. Each fowl was given 50 (plus or minus 5) embryonated eggs of the intestinal nematode, *A. lineata.* After three weeks the birds were all killed and the worms collected as described. The turkeys

TABLE I.—*A Biometrical Comparison of the Mean Numbers and Lengths of Ascaridia lineata from Chickens and Turkeys in Experiment II. (Period of Parasitism, Three Weeks.)*

	Fowls	Number of hosts	Means	Stand. Dev.	Error of mean	Difference	Ratio, $\frac{D}{E}$
Number of Worms	White Leghorn Chickens	25	6.12	7.94	1.07	4.92	4.32
	Bronze Turkeys	15	1.20	2.21	.385		
Length of Worms (mm.)	White Leghorn Chickens	25	26.07	6.27	.316	6.50	11.82
	Bronze Turkeys	15	19.57	3.68	.641		

[4] At the suggestion of Mr. H. M. Scott slight changes in the proportions of some of the food ingredients were made to make the ration better adapted for turkeys.

had an average of 1.2 worms per bird. The chickens, on the other hand, had an average of 6.12 worms, or a difference of 4.92 worms per bird. This difference was 4.32 times its probable error and therefore considered to be significant (Table 1).

On measuring the lengths of the worms it was found that those from the turkeys averaged 19.57 mm. per bird and that the worms from the white leghorns averaged 26.07 mm., a difference of 6.50 mm. As this difference was 11.82 times its probable error it is considered significant. From the results of this experiment it is concluded that both to numbers of worms (viability) and to lengths of worms (growth) the turkeys were more resistant than were the chickens. In other words, the results fail to support the contention that the chicken is an abnormal host of *A. lineata* and the inference that the turkey is the normal and, hence, less resistant host.

DISCUSSION

Recent records of the occurrence of the large intestinal roundworm in turkeys are scarce. Hassall (1896) included the various early European records of it in his check list. Vawter and Records (1928) state that "The large roundworm (*Ascaridia lineata*) is commonly found in the intestines. . . . Young turkeys are commonly infested by this worm which may be responsible for unhealthy appearance and retarded growth. Older turkeys are more resistant to the effects of this parasite." Monnig (1928) records *A. lineata* (Schneider) from the fowl and turkeys in South Africa. In these reports there is no indication as to whether the turkeys or the chickens are the more resistant to this intestinal nematode.

While Ransom's results with gapeworms indicate that the chicken is more resistant than the turkey, it is not certain that the chickens examined in the Washington market were exposed to the gapeworms. Furthermore, in the experimental tests, Ransom appears to have used but three adult turkeys. Had he used a larger number of turkeys, it is probable that the percentage of infestations would have been lowered. Whether there are constant differences in the resistance of turkeys and of chickens to the intestinal roundworm, *A. lineata,* is uncertain. With the increase of turkey production in the U. S. in recent years, it is hoped that information on the relative occurrence of the large roundworm in turkeys and chickens will be made available. So far as can be ascertained both from the natural occurrence of *A. lineata* in turkeys and chickens and from experimental infestations, there is no evidence to indicate that the turkey is the natural and less resistant host of *A. lineata.* From the present tests, white leghorn chickens appear to be greater factors in the spread of *A. lineata* than are bronze turkeys.

SUMMARY

Two experiments were carried out to compare the resistance of bronze turkeys and white leghorn chickens of the same age to the viability and growth of the intestinal nematode, *Ascaridia lineata* (Schneider). The individual birds under comparison were given the same number of embryonated eggs of *A. lineata.*

The period of parasitism in the first experiment was one month, that for the second, three weeks. In both experiments the turkeys harbored fewer nematodes than did the chickens. In the second experiment the nematodes from the turkeys were significantly fewer in number and shorter in length than were those from the chickens. There was no evidence that the turkeys are the natural hosts nor that the chickens are abnormal hosts and hence more resistant than the turkeys to this nematode.

White leghorn chickens at approximately two months of age are probably greater factors in the spread of the intestinal roundworm, *A. lineata,* than are bronze turkeys of the same age.

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AGE RESISTANCE OF CHICKENS TO THE NEMATODE *ASCARIDIA LINEATA* (SCHNEIDER)[1]

J. E. Ackert, D. A. Porter, and T. D. Beach

INTRODUCTION

Empirical evidence of greater resistance to helminthiasis in older individuals than in younger ones is abundant. Epidemiological studies, especially during the last decade, also give evidence of age resistance to many helminths, and the results of egg counts bear further testimony of the occurrence of increased resistance to certain helminths in growing hosts. Observations in 1911 by Looss pointed to resistance with increased age since in transferring larvae of *Ancylostoma duodenale* to dogs, he observed that in young animals some of the larvae were able to reach maturity. The observations of Ransom and Foster (1920) on Ascaris in pigs also show age resistance and Ransom, in 1921, observed age resistance of chickens to *Syngamus trachea*. In the same year, while studying the effects of the intestinal nematode, *Ascaridia lineata*, in chickens, Ackert and Herrick (1928) found that the birds four months of age were much more resistant to the nematodes than were the chicks one month old. Subsequent confirmations of these findings led to the studies of Herrick (1926) who found a gradual increase in the resistance of chickens to the growth of the nematode during periods of 10 days of parasitism. Beginning with chicks five days of age his results indicated that the resistance increased with age up to 103 days after which no further increase was detected.

Demonstrations of age resistance by quantitative methods have also been shown for *Ancylostoma caninum* in cats and dogs (Herrick, 1928; Scott, 1928; Sarles, 1929b); *Ancylostoma braziliense* in cats and dogs (Sarles, 1929a), and *Nippostrongylus muris* in rats (Africa, 1931). Chandler (1932) in experiments with *Nippostrongylus* found that fewer worms were recovered from rats 5 to 12 months old than from 4 to 10 weeks old, but concluded that the difference in numbers at autopsy was of doubtful significance. Graham (1932) using a modified Stoll egg count method on the nematode, *N. muris*, found rats 14 weeks of age more resistant than those six weeks old. Winfield (1933) demonstrated an age resistance of rats to *Heterakis spumosa*. He used as criteria egg counts and size and number of worms at autopsy.

The detection of age resistance by parasitizing numbers of animals with known numbers of eggs or larvae and by collecting at autopsy all

[1] Contribution No. 156 from the Department of Zoology, Agricultural Experiment Station, Kansas State College of Agriculture and Applied Science, Manhattan.

worms (including larvae and males) has been somewhat restricted. It is to augment the evidence of this character that the data of the present paper are recorded.

MATERIAL AND METHODS

The chickens used in these tests were purebred white leghorns secured as day old chicks and raised in confinement under conditions adequate for normal growth. Throughout the course of the experiments the chickens were kept on diets essentially as follows: yellow cornmeal, 45 gm.; cracked wheat, 15 gm.; oatona (steel cut oats), 17 gm.; alfalfa leaf meal, 4 gm.; meat meal, 10.5 gm.; powdered milk, 6.5 gm.; and cod liver oil, 2 gm. In selecting the groups of chickens to be parasitized the largest and smallest individuals of the hatch were rejected in order to secure a more uniform group.

The nematode (*A. lineata*) eggs used for parasitizing were obtained from live worms and cultured at 30° C. in water containing a trace of 2 per cent formalin. The embryonated eggs were counted with the aid of a compound microscope and mechanical stage and fed to the individual chickens in corn meal pellets. The young nematodes were given three weeks in which to develop, because at the end of this time the worms have withdrawn from the intestinal wall where they have been partially buried and have taken up their abode in the lumen of the intestine (Ackert, 1923). At the close of the experiment, the birds were killed, the intestinal contents flushed into a flask by the hydraulic method of Ackert and Nolf (1929) and the material preserved in 10 per cent formalin until isolation and measurement of the nematodes. The criterion for determining differences in age resistance was the lengths of the *A. lineata* removed from the chickens. The measuring of these nematodes was accomplished by throwing a shadow (magnified six diameters) of the worm upon ground glass of a photographic bellows, tracing the enlarged shadow of the worm on thin paper and determining the length by the aid of a calibrated tracing wheel. This makes it possible to determine accurately the lengths of all worms including those that assume coiled or twisted positions.

EXPERIMENTAL DATA

The data here presented are from nine experiments. In each experiment the individual chickens after being raised helminth-free for several weeks, were fed, on the same day, 50 plus or minus 5 eggs, or 300 plus or minus 10 eggs as described. After being parasitized the groups of chickens were continued on the same adequate diets for a period of three weeks when all chickens in the experiment were killed on the same day, the intestines being removed and the contents flushed out immediately

after decapitation of the bird. In this way the worms were removed before the mucous glands poured out their opaque secretions.

Results from feeding 50 eggs. The chickens in groups I to V were parasitized by being fed 50 eggs of the nematode, *A. lineata*. In table 1 it may be seen that group I consisting of 31 chickens which were ex-

TABLE 1.—*A biometrical comparison of the mean lengths of A. lineata resulting from egg-doses of two sizes administered to chickens of different ages. (Period of parasitism three weeks.)*

Group	Number of hosts	No. of worms measured	Mean length of *A. lineata* (mm.)	Stand. Dev.	Dif. of means (= D)	Probable error of Dif. (= E)	Ratio, $\frac{D}{E}$	Group having larger mean
			Fed 50 eggs					
I–58 day vs. ..	31	347	26.78 ± 0.23	6.49	6.75	0.328	20.57	58 day
II–63 day vs. ..	89	557	20.03 ± 0.23	8.04	7.31	0.340	21.51	63 day
III–71 day vs. ..	45	377	12.72 ± 0.25	7.26	1.29	0.434	2.96	71 day
IV–84 day vs. ..	35	98	11.43 ± 0.35	5.12	0.45	0.550	0.81	114 day
V–114 day	19	82	11.88 ± 0.42	5.65				
			Fed 300 eggs (approx.)					
VI–45 day vs. ..	20	309	21.19 ± 0.23	5.96	5.09	0.297	17.10	45 day
VII–63 day vs. ..	94	786	16.10 ± 0.19	7.99	4.14	0.564	7.35	63 day
VIII–71 day vs. ..	10	115	11.96 ± 0.53	8.45	8.03	0.708	11.33	71 day
IX–93 day	39	157	3.93 ± 0.47	8.70				

amined at the age of 58 days yielded a total of 347 worms. The mean length of these nematodes was 26.78 mm. When this result is compared with that from group II (63 days old), whose nematodes averaged 20.03 mm., a difference of 6.75 mm. in length results. This difference is 20.57 times its probable error. Since this difference is at least four times its probable error it doubtlessly lies beyond the limits of experimental error, and hence, is not due to chance variation. It is therefore considered significant. In the case of group II, five days of increased age were sufficient to cause a marked limitation in the growth of the nematodes.

Group III consisted of 45 chickens examined when they were 71 days old. They yielded 377 *A. lineata* whose mean length was 12.72 mm. This length compared with the 20.03 mm. from the younger group (II) gave the significant difference of 7.31 mm. which is 21.51 times its probable error. In the case of group III a difference of eight days in age resulted in a marked inhibition in the growth of the worms in the older chickens.

Continuing the experiments in which 50 eggs were fed to each chicken a test was made on group IV, the chickens having been examined

at 84 days of age. From this group 98 *A. lineata* were collected. Their mean length was 11.43 mm. as compared with 12.72 mm. for group III (71 days old). The difference in length between these groups was 1.29 mm., which was only 2.96 times the probable error and therefore probably not significant. Group V consisting of 19 chickens 114 days of age yielded 82 nematodes with a mean length of 11.88 mm., just slightly longer (0.45 mm.) than those from the 84-day group. This variation could be expected as no further increase in resistance after 113 days of age was observed by Herrick (1926). The results from the last two comparisons indicate that under the conditions of these experiments the rate of acquiring age resistance slackened shortly after the chickens became 71 days of age. This is in accord with the results of tests made by Ackert (1930) who found chickens 10 weeks of age to be very resistant both to the growth of *A. lineata* and to effects of these parasites upon the hosts. Similarly, Ackert, Graham, Nolf and Porter (1931) found chickens 10 weeks of age to be markedly more resistant to the viability and growth of *A. lineata* than were the birds seven and six weeks of age. From these five tests in which each chicken was fed 50 embryonated eggs of the parasite (*A. lineata*) the resistance of the chickens to the growth of the nematode continued to increase significantly until the chickens were 71 days of age (fig. 1). While there was an indication of a slight increase beyond that age the difference lay within the limits of experimental error.

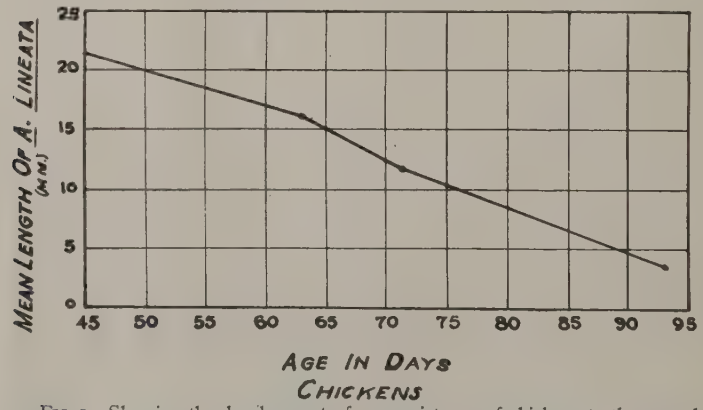

FIG. 1. Showing the development of age resistance of chickens to the growth in length of the intestinal nematode, *Ascaridia lineata* (Schneider). Each chicken given 50 embryonated eggs of *A. lineata*. Period of parasitism three weeks.

Results from feeding 300 eggs. In the second series of experiments each chicken was fed 300 embryonated eggs of the nematode, *A. lineata*,

the period of parasitism having been three weeks as in the previous experiments. Group VI consisting of 20 chickens was 45 days old when examined. It yielded 309 worms which averaged 21.19 mm. in length. These results were compared with those from group VII consisting of 94 chickens 63 days of age. On examination of group VII 786 worms were isolated. These had an average length of 16.1 mm. which compared with the 21.19 mm. (average length of group VI) had a significant difference of 5.09 mm., again showing marked age resistance. Tests were next made on chickens 71 days old (group VIII). The 115 nematodes collected had a mean length of 11.96 mm. which when compared with the 16.1 mm. average from group VII gave a difference of 4.14 mm., which was significant (table 1). Of the chickens fed 300 eggs the last test was made upon group IX, 93 days of age. From them were collected 157 *A. lineata* which averaged 3.93 mm. in length. This length compared with that from group VIII gave a difference of 8.03 mm., which was 11.33 times its probable error and is therefore considered significant.

From these experiments it may be seen that the resistance of the chickens to the growth of the nematodes gradually increased with the

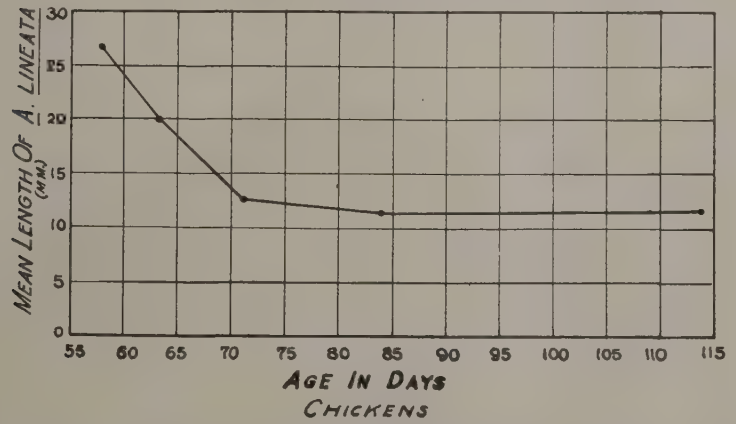

FIG. 2. Showing the development of age resistance of chickens to the growth in length of the nematode, *A. lineata*. Each chicken given 300 embryonated eggs of *A. lineata*. Period of parasitism three weeks.

age of the chickens (fig. 2). As the groups of chickens become older the lengths of the worms removed were shorter. In other words during the three weeks of parasitism the worms growing in the 45-day group of chickens were able to attain an average length of 21.19 mm.; in the 63-day group, 16.1 mm.; in the 71-day group, 11.96 mm., and in the 93-day group of chickens the nematodes were able to grow only 3.93

mm. in length affording marked evidence of age resistance of chickens to the growth of the intestinal nematode, *A. lineata.*

DISCUSSION

Although such resistance has been observed in many host-parasite relationships, the lack of it has been noted by some investigators. Ransom found that chickens possessed a very definite age resistance to the gapeworm, *Syngamus trachea,* but that old trukeys could harbor an experimental infestation as well as younger birds. Sandground (1928) failed to obtain evidence of age resistance in experimental infestations of dogs and cats with *Strongyloides stercoralis.*

In an able review of resistance, Sandground (1929) says, "The high correlation that exists between the exhibition of age resistance and a greater or lesser condition of abnormality in the species relation of the parasite and host is of significance, and that it must necessarily be represented as an important, if not critical, feature in any explanation of the phenomenon." This deduction appears to have been based on evidence that an age resistance is shown by dogs to *A. duodenale* and by chickens to *Syngamus trachea,* both dogs and chickens being considered as abnormal hosts for these respective parasites. The normal hosts (man and turkeys) appeared not to show an age resistance against the respective parasites. Sandground further states, "From the details of the life history bared by the studies of Herrick and briefly mentioned above it is difficult to avoid the conclusion that the behavior of *A. lineata* in the chicken exhibits all the characteristic marks of a parasite living in an environment that is at its best only partially favorable to its existence." As Sandground considers the chicken an abnormal host for *A. lineata,* as this nematode was described originally from a specimen harbored by a turkey, and as the latter has been considered to be the normal host of *Syngamus trachea,* the reader is left to infer that the turkey is the normal and less resistant host of *A. lineata.*

That this view is probably untenable is shown by the work of Ackert and Eisenbrandt (in press) in a study of the comparative resistance of chickens and turkeys to *A. lineata.* Two tests were carried out. In the first test in which 4 chickens and 4 turkeys were used, the chickens harbored more worms than the turkeys, and in the second test in which 25 chickens and 15 turkeys of the same age were used, the chickens had significantly more and longer *A. lineata* than did the turkeys. These results show that the white leghorn chickens were more favorable hosts for *A. lineata* than were the bronze turkeys, and fail to support the contention that the chicken is an abnormal and hence more resistant host of this intestinal worm. That age resistance cannot be explained on the basis of an abnormal host-parasite relationship is held by Winfield

(1933) who experimentally infested a domesticated variety of rats, *Epemys norwegicus,* with eggs from the intestinal nematode, *Heterakis spumosa,* isolated from a wild strain rats of the same species.

The more rapid growth of the fowl nematode, *A. lineata,* in young chickens than in older ones has an interesting parallel in the recent work of Bahrs (1931) who found that Planaria fed on intestinal mucosae of young rabbits; also that the Planaria grew faster on mucosae of well fed than of starved rabbits. This would tend to explain the differences in the size of the nematodes on the basis of a greater growth-promoting power of the mucosae of young and well fed animals. On this hypothesis it would be expected that the *A. lineata* would thrive better in the intestines of well fed chickens than in those of chickens whose diet lacked an essential accessory. Just the converse resulted in repeated experiments by Ackert, McIlvaine, and Crawford (1931) who found significantly more and longer *A. lineata* in chickens whose diet lacked vitamin A than in the control chickens to which this vitamin was supplied. It should be noted, however, that *A. lineata* appears not to require vitamin A (Ackert, 1930). But in experiments by Ackert and Beach (1933) the group of chickens on the best diet had the fewest and shortest *A. lineata.*

The hypothesis of resistance being in the nature of an inhibiting mechanism seems to be more plausible. Carrel and Ebeling (1921) showed the presence of an active growth-inhibiting substance in the blood serum of chickens. The inhibiting power increased with the age of the chicken. They state, "The action of age on the serum is characterized, not by the decrease of an accelerating factor, . . . but by the increase of an inhibiting factor." That an inhibiting factor which becomes available to *A. lineata* is present in chickens is indicated by the work of Ackert (1925) and of Porter and Ackert (1933) in whose tests the resistance of chickens to *A. lineata* was lowered by blood letting. Whether the resistance mechanism is in the form of a fluid that passes from the intestinal wall into the lumen, whether it is a deposit in the mucosa, whether the cellular mucosa becomes tougher, or whether the resistance mechanism is of some other nature is unknown.

SUMMARY

1. Nine tests involving 382 chickens were carried out to determine the degree of age resistance of chickens to the intestinal nematode, *Ascaridia lineata* (Schneider). The chickens were pure bred, single comb white leghorns secured as day old chicks from tested commercial hatcheries; they were raised helminth-free until parasitized with embryonated eggs of the nematode, *A. lineata.* After three weeks of para-

sitism, the chickens were killed and the nematodes isolated, counted and measured.

2. The basis for judging the degree of host resistance to the nematodes was the average worm length per group of chickens.

3. In the first five tests each chicken was fed 50 eggs and in the remaining four tests each bird was given 300 eggs.

4. The tests in which 50 eggs were fed were made on groups of chickens examined at the following ages: 58 days, 63 days, 71 days, 84 days, and 114 days; those to which 300 eggs were fed were examined at the ages of 45 days, 63 days, 71 days, and 93 days, respectively.

5. When 50 eggs were fed, the worms were found to be significantly shorter in the 63-day group than in the 58-day group of chickens, and those in the 78-day group were markedly shorter than the worms from the 63-day group. The worms from groups 84 and 114 days of age were shorter than the previous ones, but not significantly so.

6. When 300 eggs were fed, significantly increased resistance was demonstrated by the worms from the 63-day group as compared with those from the 45-day group. Likewise, the worms from the 71-day group were markedly shorter than those from the 63-day group; and the worms from the 93-day group were significantly shorter than those from the 71-day group of chickens.

7. Thus, the results of these experiments demonstrate a marked increase in the resistance of chickens to the growth of the intestinal nematode, *A. lineata*; starting with chickens 45 days of age, significant differences in the lengths of the nematodes were noted up to 93 days of age when the maximum resistance is ordinarily reached.

8. The explanation of these results and of those from closely related experiments does not lend itself to the abnormal host theory nor to that of a growth-promoting power. The most plausible explanation is that as the chicken grows older its body develops more potent growth-inhibiting factors which react against the development of the nematodes.

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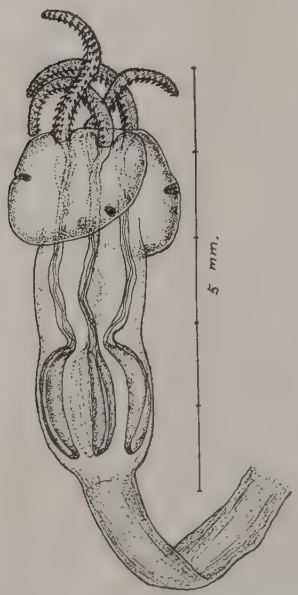
RESEARCH NOTES

A NEW TETRARHYNCHID LARVA FROM GALVESTON BAY

From time to time fishermen complain that the spotted sea trout, *Eriscion nebulosus,* caught in Galveston Bay, is "wormy," particularly during the winter months. A catch of about twenty of these fish was brought to this laboratory in December, and four or five were found to have the flesh infested by Tetrarhynchid larvae; two of the fish contained considerable numbers of these larvae, at least twenty or thirty of them, their long caudal appendages, in some cases 17 cm. long, twined back and forth through the musculature of the back and sides. Examination showed that the majority of the larvae belonged to a hitherto undescribed species of *Otobothrium.* A description of the new species follows.

Otobothrium robustum n. sp. Cysts up to 17 cm. long, consisting of an elongate egg-shaped anterior enlargement containing the scolex, and a very long narrow tail-like portion; anterior enlargement about 7 mm. long and 2.5 to 3 mm. broad. Scolex and neck, freed by rupturing cyst, 3 mm. to 4.4 mm. long, followed by a flattened tail-like appendage up to 16 cm. or more in length and about 600 to 800 μ in breadth. Bothria two, heart-shaped, each with a pair of ciliated pits on the lateral margins, about midway between anterior and posterior extremities of the bothria. Bothria 1.3 to 1.7 mm. long and about 1.3 to 1.4 mm. broad.



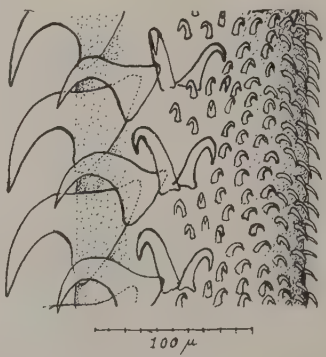


Fig. 1. *Otobothrium robustum* n. sp., with proboscides almost fully extended. Specimen somewhat flattened.

Fig. 2. Small portion near middle of proboscis showing arrangement of hooks. Each whorl of large hooks on one side of the proboscis consists of two sets of four hooks each, only set of which is visible in this view.

Diameter just behind bothria in somewhat flattened specimens 1.2 o 1.4 mm., this diameter being practically the same back to the junction with the tail. Proboscides about 2 mm. long, tapering from a basal diameter, exclusive of the hooks, of about 150 μ to a diameter of about 90 μ near the tip. Proboscis provided with large claw-like hooks on one side and very small hooks on the other (Fig. 2). Larger hooks in successive whorls about 70 μ apart; in each whorl a pair of large hooks measuring 110 to 115 μ from tip to farthest extremity of base; these hooks followed on each side by three successively smaller hooks each slightly more distal in the whorl than the preceding one, thus forming a very broad "V" converging to the pair of largest hooks. Other half of proboscis covered by very numerous curved spine-like hooks about 16 to 20 μ in length, not set in whorls but tending toward irregular diagonal rows. Proboscis sheaths almost straight, without spirals. Contractile bulbs banana-shaped, 1 to 1.4 mm. long and 220 to 280 μ broad, well separated from each other.

Host: *Eriscion nebulosus.*

Location: Musculature.

Locality: Galveston Bay.

Type specimen: U. S. Nat. Mus. Helm. Coll. No. 8825.

This species differs from all other members of the genus except *O. balli* Southwell, 1929, in having the ciliated pits midway on the lateral margins of the bothria instead of at the tip. From *O. balli* it differs strikingly in the proportions of the parts of the head and in the size and arrangement of the proboscis hooks. In the arrangement of hooks, and in other respects, it has some resemblance to *O. linstowi* Southwell, 1912, but differs in the position of the ciliated pits, the shorter and differently shaped contractile bulbs, the larger number of small spine-like hooks, and in the absence of spirals in the proboscis sacs.

There is no reason to suppose that fish which contain a few of these larvae in the flesh would not be perfectly wholesome as food, since the adult stages of these worms are reached in elasmobranch fishes, in this case very likely a ray.—Asa C. Chandler, *Biology Laboratory, Rice Institute, Houston, Texas.*

OESOPHAGOSTOMUM LONGICAUDUM GOODEY, 1925, A SYNONYM OF *OESOPHAGOSTOMUM QUADRISPINULATUM* (MARCONE, 1901)

In 1929, Goodey reported a new species of nodular worm from swine in New Guinea and named it *Oesophagostomum longicaudum.* Following the publication of Goodey's paper, Schwartz (1925) pointed out that this parasite had rather a wide geographical distribution, having been collected in the United States, Philippine Islands, Tonkin (Indo-China), Fiji Islands and China. Some of the outstanding characters of this species, according to Goodey, are as follows: Anterior end of esophagus swollen; spicules 0.91 to 0.95 mm. long; vulva 380 to 460 μ from anal opening; tail long and tapering, 400 to 460 μ long.

In a paper published in 1901 (La Riforma Veterinaria, Napoli, v. 4 (1), Gennaio, pp. 3–26), G. Marcone reported that he had collected from swine, nematodes of the genus *Oesophagostomum,* which differed from *O. dentatum* in having only 4 cephalic papillae instead of 6 cephalic papillae as reported by previous authors. Apparently, Marcone failed to note that the 6 cephalic papillae reported by other authors consisted of the 4 long and pointed submedian cephalic papillae and 2 smaller lateral papillae or amphids. Because of this oversight, Marcone proposed a new variety for the specimens which he collected and stated "Sicchè questa varieta si potrebbe denominare *oesofagostoma dentatum quadrispinulatum.*" Marcone's description and figures of this variety correspond closely to Goodey's description of *O. longicaudum.* Some of the measurements given by Marcone are as follows: Total length of males and females, 8 to 10 mm. and 11 to 14 mm., respectively; spicules 800 μ long; vulva 460 μ from anal opening; tail 490 μ long. Marcone's illustrations show that the anterior end of the esophagus is swollen

and that the tail of the female is long and tapering, characters which, according to Goodey, are diagnostic of *O. longicaudum*.

Due to the fact that Marcone's (1901) description of this nodular worm corresponds to that given by Goodey (1925), the name used by the former has priority under the rules of zoological nomenclature. The writer proposes to elevate Marcone's variety to specific rank. The correct name of the parasite in question is *Oesophagostomum quadrispinulatum* (Marcone, 1901); *Oesophagostomum longicaudum* Goodey, 1925, becomes a synonym of *O. quadrispinulatum*.—Joseph E. Alicata, *Zoological Division, Bureau of Animal Industry, U. S. Department of Agriculture, Washington, D. C.*

THE TICK *ORNITHODOROS TALAJE* IN WISCONSIN HOMES

During the fall of 1930 two ticks of the genus *Ornithodoros* were brought to the Department of Zoology for identification. The man who brought in the specimens was very reluctant to give any information in regard to the place where the ticks were found. He eventually stated, however, that the ticks were found on the wall of a room in an old hotel and that they were annoying the guests. The ticks were alive when brought into the laboratory but were not engorged. These and other ticks taken from the places described were sent to Dr. Allen McIntosh, Zoological Division, Bureau of Animal Industry, U. S. Department of Agriculture, Washington, D. C. They were identified by him as *Ornithodoros talaje*.

Since 1930 ticks of this species have been brought to the Zoological Department from a men's club, a private home, and four rooming houses. The ticks from the men's club were found crawling about on the walls of a third floor room which was next to the attic. No ticks were found in other rooms of the club nor were other specimens found in the room from which they were first taken. No one seemed to have been bitten by the ticks and none of the eight specimens found were engorged. The presence of the ticks could not be explained by members of the club.

The ticks found in the rooming houses were brought in from October to March. In most cases only four or five specimens were sent in and little could be learned about the conditions under which the ticks were taken. One student said they were numerous in his rooming house and brought in twenty living specimens as evidence. He assured me, however, that no one was being bitten and asked that nothing be said or done about the condition.

The person bringing the ticks from the Madison home has cooperated in every way possible. The home referred to is a rambling old mansion and has been occupied by a wealthy and fastidious family. Owing to the age and construction of the house, fumigation has been considered by experts to be impractical. As a result, the family has attempted to free the house by local treatments of one kind or another, and a constant search has been made for specimens. The ticks were first found on the walls of a third floor room nearest the attic. Later they were found in all parts of the house; on the walls, stair cases, picture frames, and many other places. They were usually found crawling about during the day. No one in the house seemingly has been bitten by the ticks and none of the specimens received have been engorged, although some specimens that were destroyed were said to contain blood. Over seventy-five specimens have been brought to the laboratory and it has been estimated that approximately twice that number has been destroyed. They have been found during every month of the year and, although they have been vigorously fought ever since their discovery, they have been present for a period considerably over three years.

Members of the family occupying this tick-infested house are often in the south and many of their guests have come from the south. Furthermore, some foods such as coffee have been received directly from wholesale houses and have been received in the original wrappings. Under these circumstances it would

not be difficult to explain the presence of a few specimens of *Ornithodoros talaje* in a Wisconsin home but the explanation for their continued presence can not be given at this time.—C. A. Herrick, *Departments of Zoology and Veterinary Science, University of Wisconsin, Madison, Wis.*

MINNESOTA RECORDS OF *ORNITHODOROS TALAJE*

The note by Dr. C. A. Herrick relative to the occurrence of the tick, *Ornithodoros talaje,* in Wisconsin, is of special interest to me since this "tropical" tick has also been taken in Minnesota under conditions which offer an explanation as to its presence.

In the first instance, the late C. W. Howard received a male specimen on December 28, 1915, from Le Sueur, Minnesota. On investigation it was found that it had dropped from the ceiling of an upstairs picture-framing shop. The owner had been receiving glass packed in hay, in boxes, from Oklahoma. Four months later another specimen was received, from this same shop.

On April 5, 1933, I received a female specimen from a residence in Lake City, Minnesota. The sender added that he had found several of them in the bathroom, in the soiled clothes hamper, and in one of the beds. About a month later he brought to my office eight additional specimens, four of which were nymphs and four adults.

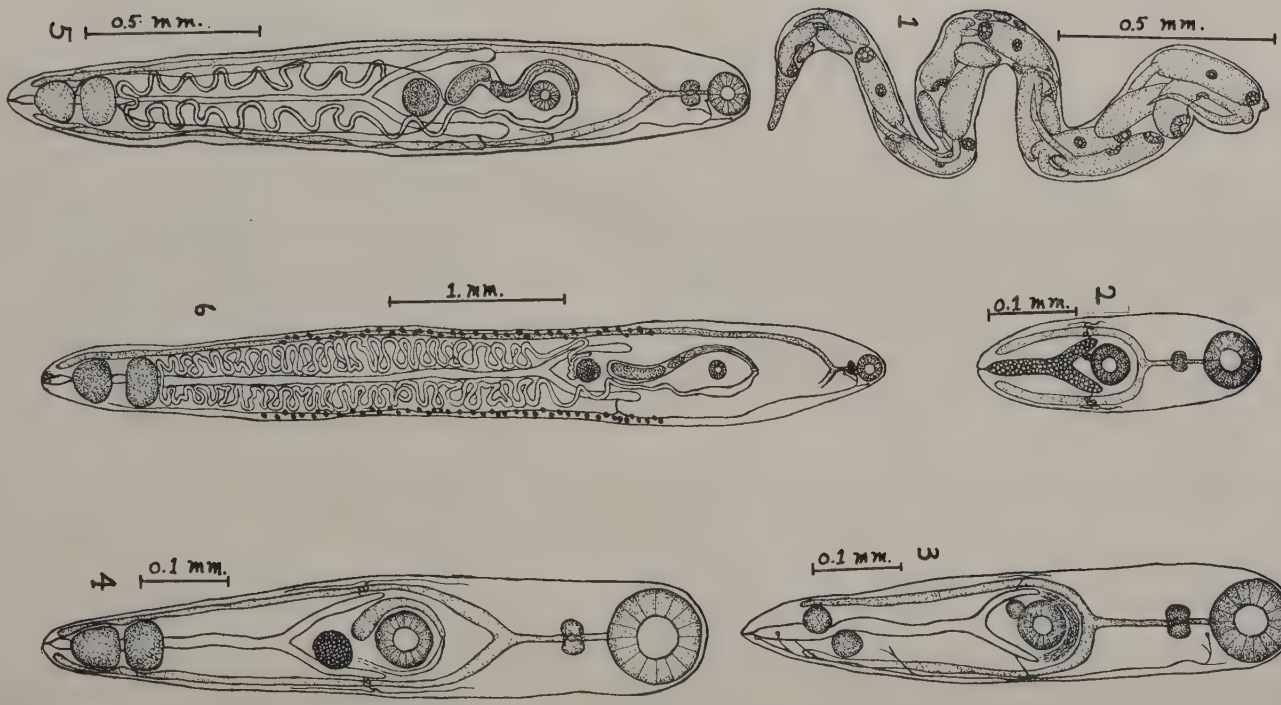
In this case, it developed that two years previously the family had been in New Mexico, and had brought back with them several Navajo rugs and some carefully packed pottery. Rats and mice were present in the house. As these rodents are readily attacked by *O. talaje,* it seemed very probable that they served as a source of food. Unfortunately, I could get no direct evidence of this and argasid ticks are notorious for their ability to withstand starvation.

In neither the Wisconsin or in the Minnesota cases is there evidence of the ticks having fed on man. As the bite of this species is well known to be very painful, the presence of even small numbers in residences possesses something more than a technical interest.—Wm. A. Riley, *University of Minnesota.*

A NOTE ON THE RELATIONSHIP OF THE TELORCHINAE AND THE RENIFERINAE

There has been a great deal of confusion in the classification of the telorchids. Various authors have suggested that they were related to at least four different families and it has even been suggested that they should have the rank of a family. Fuhrmann (1928, W. Kukenthal, Handbuck der Zoologie, Trematoda, p. 112) included the genera of the Telorchinae in the list of genera belonging to the family Plagiorchidae. Mehra (1931, Parasit., 23: 157–178) followed Perkins (1928, Parasit., 20: 336–356) in making a subfamily of the group, but definitely placed the Telorchinae in the family Plagiorchidae. The similarity of the life cycles of *Cercorchis medium* (McMullen, 1934, Jour. Parasit., 20: 248–250) and *C. robustus* (Krull, 1935, Proc. Helm. Soc., 2: 65) to those of other Plagiorchidae indicates that this is the proper relationship. A comparison was made of the developmental stages of the former species with those of the other members of the Plagiorchidae and a striking resemblance to the Reniferinae was found.

Seven life cycles of members of the Reniferinae, representing six genera, were found in the literature (McCoy, 1928, Jour. Parasit., 14: 207–228; Ingles, 1933, Univ. Cal. Pub. Zool., 39: 163–178; Talbot, 1933, Parasit., 25: 518–545). In the latter paper Talbot pointed out that similarity in the larval characteristics indicates that the Reniferinae is a natural group. The larval stages in the life cycle of *C. medius* are very similar to those described for the members of the Reniferinae. Since the relationship is not so evident in the adults this demonstrates, once more, the importance of studying the larval characteristics.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3
0.1 mm.

The mature daughter sporocyst (Fig. 1) of *C. medius* is similar in size and general organization to this stage of the Reniferinae. The similarity of the cercaria of this species (see fig. 3, 1934, Jour. Parasit., 20: 250) to those described for the Reniferinae is very clear. In fact there is no greater difference between the cercaria of *C. medius* and those of the members of the Reniferinae, than between the cercariae of the members of the different genera of this subfamily. The most striking likeness is in the excretory system. The excretory bladder in both *C. medius* and the Reniferinae is Y-shaped with long arms extending partially around the acetabulum. The main collecting tubes are attached laterally and posterior to the anterior tip of the arms of the bladder. The stylet glands are about the same in number and are situated in the same region of the body. The digestive system differs from those found in the cercariae of most members of the Reniferinae by having intestinal ceca that extend to the posterior end, but this is also found in the cercaria of one species, *Caudorchis eurinus* Talbot, 1933. The metacercaria (fig. 2) of *C. medius* has the same general characteristics seen in the body of the cercaria, and also is similar to the metacercariae of the members of the Reniferinae.

The similarity persists in the very immature stages. Immature specimens of *C. medius* (fig. 3) recovered from an abnormal host, a gartersnake, one week after infection show the characteristics of the metacercaria. There is, however, an increase in size and the developing gonads are seen in a position resembling that of most other plagiorchids. Their arrangement is particularly like that found in the early stages of *Lechriorchis primus* and *Caudorchis eurinus*, as shown by Talbot (1933).

From this point in the development of *C. medius* the differences from the Reniferinae become more and more striking. The excretory system in the adult is the only system which indicates that there is a relationship. The flukes become very much elongated, the testes are in the posterior end and tandem, the cirrus is very much elongated and the uterus separates the testes and the ovary.

The immature flukes (fig. 4) that were recovered one week after feeding a turtle, the normal host, show the typical telorchid arrangement of the reproductive organs. In specimens (fig. 5) recovered from a turtle, four weeks after infection, the development has reached the point where egg production is ready to begin and those (fig. 6) obtained from an experimental animal, six months after infection, are adults.

Since these two groups of trematodes show such striking similarities in the larval stages it is evident that they are related. The question arises whether they should be combined in one subfamily or be separated into two subfamilies as at present. Since both subfamilies contain relatively large numbers of genera and species, and the adults are strikingly different the retention of the separate groups would seem to be desirable. As knowledge of the life cycles of the Plagiorchidae increases it becomes evident that this whole family needs revision on the basis of relationships shown by the structure of the excretory system and larval stages. When such a revision becomes possible some arrangement should be made to express the relationship of the Telorchinae and the Reniferinae.—Donald B. McMullen, *Department of Helminthology, Johns Hopkins School of Hygiene and Public Health.*

Fig. 1. Sporocyst of *Cercorchis medius*, from *Physella entegra*, × 100.

Fig. 2. Metacercaria of *Cercorchis medius* from an experimentally infected tadpole, × 200.

Fig. 3. Immature fluke from a gartersnake, 7 days after feeding, × 200.

Fig. 4. Immature fluke from a turtle, 7 days after feeding, × 200.

Fig. 5. Immature fluke from a turtle, one month after feeding, × 80.

Fig. 6. Adult *Cercorchis medius* from experimental turtle, 6 months after feeding, × 40.

IS THE CAPUCHIN MONKEY REFRACTORY TO INFECTION WITH *ENDAMOEBA HISTOLYTICA*?

In February, 1934, I received four adult *Cebus capucinus* (3 males and 1 female) from the Psychology Department of this institution. Repeated attempts to infect these animals with *Endamoeba histolytica* either by cysts or trophozoites failed completely. Material for infection was obtained both from man and from *Macacus rhesus*; attempts to infect were made by mouth and by rectal injections. In all, eleven different feedings and injections were attempted over a period of nine months. Hegner and Holmes (Amer. Jour. Hygiene 3: 252–263. 1923) reported an infection of *Cebus variegatus* with "three species of amoebae"; no infection of *Cebus capucinus* with *Endamoeba histolytica* has been recorded either naturally or artificially to the writer's knowledge. In order to check the method of infection, an adult *Macacus rhesus* was obtained, cleared of amoebae by vioform, and the infection experiments repeated on all five animals. Only the rhesus became infected; the animals have since all been sacrificed; ulceration was found only in *Macacus rhesus*.—Gordon H. Ball, *University of California at Los Angeles.*

A STUDY OF THE MOISTURE REQUIREMENTS OF THE EGGS OF THE CHICKEN ASCARID, *ASCARIDIA GALLI*

The exact relative humidity at different temperatures in which worm eggs will develop has been determined for several species of the common ascarids (Otto, 1929, Amer. Jour. Hyg., 10: 521–525; Owen, 1930, Univ. Minn. Agric. Exper. Tech. Bull., No. 71; Onorato, 1932, Amer. Jour. Hyg., 16: 266–287; Spindler, 1929, Amer. Jour. Hyg., 10: 476–496). No such information seems to have been published concerning the eggs of the common chicken ascarid, *Ascaridia galli*. Accordingly the moisture requirement of this form were studied utilizing the technic of the above cited authors.

In a relative humidity of 40 to 50 per cent at 22° C. only an occasional egg developed beyond the morula stage but none beyond the tadpole stage. By the end of 8 days all were disintegrated. Eggs kept in a relative humidity of 77 to 82 per cent at 22° C. remained viable for from 2 to 4 weeks, but rarely developed beyond the morula stage. Eggs kept in a relative humidity of 82 to 86 per cent at 22° C. developed more slowly than those in water, but reached embryonation with only 4 per cent disintegration. Eggs kept in a saturated atmosphere at 30° C. developed exactly as did those in water at 30° C. The eggs of *Ascaridia galli* are apparently far less resistant than those of the horse and dog ascarids, *Parascaris equorum* and *Toxocara canis*, which will develop in relative humidities as low as 40 to 50 per cent and 77 per cent, respectively, at 22° C. (Otto, l. c.). They have apparently about the same moisture requirement as those of the human and pig ascarid, for the chicken ascarid eggs did not survive an average relative humidity below 81 per cent at 22° C., while those of the human and pig ascarid would not survive below a minimum relative humidity of 80 per cent at 22° C.—Anne McRae, *Johns Hopkins University, School of Hygiene and Public Health.*

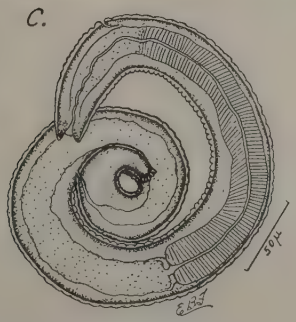
LOMASOMA, NEW NAME FOR *LOMAPHORUS*, MANTER, 1934 (TREMATODA)

Mr. Paul H. Oehser, of the Smithsonian Institution, has kindly pointed out to me that the genus name *Lomaphorus* Manter, 1934 (Carnegie Institution of Washington, Publication No. 435, p. 276) is a homonym of *Lomaphorus* Ameghino, 1889. The name *Lomasoma* is proposed to replace *Lomaphorus* for this genus of trematodes (of the family Fellodistomidae). The names of the three species become *Lomasoma wardi* (Manter) n. comb., *Lomasoma monolenei*

(Manter) n. comb., *Lomasoma gracile* (Manter) n. comb. These trematodes occur in deep-water marine fish of the Gulf of Mexico.—H. W. MANTER, *University of Nebraska.*

ENCYSTED SPIRURID LARVAE FROM THE CAT FLEA, *CTENOCEPHALIDES FELIS*

One of several fleas, *Ctenocephalides felis,* from the domestic cat, collected by the writer several years ago at Grand Island, Nebraska, was found to harbor 4 spirurid larvae spirally coiled and encysted within the body (fig. A). The cysts (fig. B) were more or less spherical, about 130 to 170 μ in diameter. Measure-



(A) Flea, showing encysted larvae.
(B) Cysts enclosing larvae.
(C) Spirurid larva dissected out of a cyst.

ments of one larva dissected out of the flea (fig. C) was as follows: Length, 625 μ; width, 38 μ; excretory pore 79 μ from anterior end of body; length of buccal cavity, about 34 μ; length of esophagus, 275 μ; length of tail, 55 μ. The cuticle presented very prominent transverse striations. The anterior extremity of the larva showed dorso-ventral cephalic elevations, and the posterior extremity was provided with a group of digitiform cuticular processes. The oral opening led into a short buccal cavity which was followed by an anterior slender proesophagus about 80 μ long, and a posterior slightly wider postesophagus about 195 μ long.

Johnston (1913) reported the findings of larval spirurids encysted in the rat fleas *Xenopsylla cheopis* and *Ceratophyllus fasciatus* collected in Australia, and stated that there was a possibility that these nematodes were the larvae of *Protospirura muris*, a spirurid parasitic in rats. According to Johnston, these larvae were 430 μ long and resembled those of *Habronema muscae*. The length of the larvae found by Johnston does not correspond to that given by Marchi (1871) who reported larvae of *P. muris* encysted in the body of the meal worm; these larvae were from 1.01 to 1.6 mm. long. There is a possibility that the larvae reported from the rat flea by Johnston are identical with or closely related to the larvae found by the writer in the cat flea, although the larvae from the rat flea were apparently smaller than those from the cat flea. The larvae from the latter possibly represent developing stages of a spirurid parasitic in cats or dogs.—Joseph E. Alicata, *Zoological Division, Bureau of Animal Industry, U. S. Department of Agriculture, Washington, D. C.*

THE EXTRA-CORPOREAL HATCHING OF ASCARIS EGGS

The hatching of ascaris eggs outside of the body has been observed by many workers under a variety of conditions. It has been observed in water, sand, and charcoal cultures by Kondo (1920, Jour. Med. Assoc. Formosa, 211: 630–633; 1922, Japan Med. World, 2: 112); Asada (1921, Japan Med. World, 1: 8) and others; in alkaline digestive fluids (Wharton, 1915, Phil. Jour. Sci., Sec. B, 10: 19–25); in pancreatic fluid (Martin, 1913, Annales des Sci. Nat., Zoology 17–18); upon transfer from acid to alkaline media (James and Martin, 1906, Compte Rend. Acad. d. Sci., 143: 67–70); in 0.2 per cent HCl and 0.2 per cent $NaHCO_3$ (Ohba, 1923, Japan Jour. Zool., 1: 121–122) and under many other specific conditions. A variety of explanations have been offered for the hatching. The last named worker felt that only very old eggs would hatch outside of the body. Wharton (l. c.) suggested that the action of algae on sand cultures may have been important in producing the hatching. Many of the workers felt that some simulation of normal digestive tract was necessary for hatching.

The writer has had generally unsatisfactory results in an attempt to hatch eggs under a variety of conditions reported to be conducive to hatching outside of the body. In these experiments eggs of the pig ascaris, *Ascaris lumbricoides*, were used. Eggs were not considered infective and therefore suitable for experimentation until they had been incubated at least *as long* and under the same conditions (usually in 2 per cent formalin at room temperature) *after reaching* the vermiform stage as was required to reach the vermiform stage (Stoll, 1933, Jour. Parasit., 20: 126; Alicata, 1934, Proc. Helm. Soc. Wash., 1: 12). Such eggs are termed infective in this paper. Infective eggs kept in 2 per cent formalin at room temperature for two years never showed more than 1 or 2 per cent hatched larvae at any one time and only 31 per cent still alive. Subjecting these eggs to temperatures of 30 and 37° C. for a month did not cause hatching. Both newly infective and old eggs failed to show more than 1 or 2 per cent hatching when transferred from 0.2 per cent HCl to 0.5 per cent Na HCO_3 at either 30 or 37° C. Three weeks in artificial gastric juice (0.7 per cent HCl and 1 per cent pepsin) failed to cause hatching. Alternate cooling and warming (0° C. to 40° C.) also failed. Despite these negative results the writer and others in the Department found evidence of "spontaneous" hatching in some cultures from time

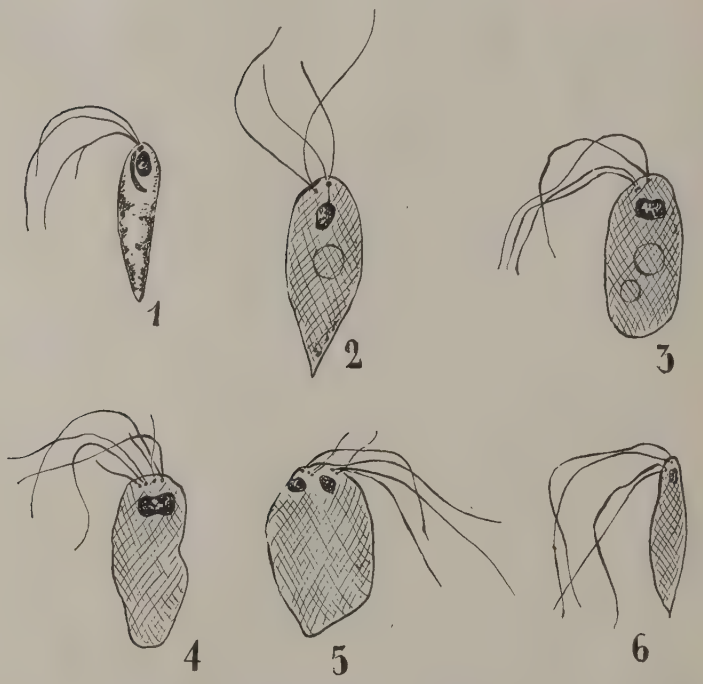
to time. In one such case a culture of eggs on the surface of sand contained many hatched larvae at the end of an automobile trip of several hundred miles whereas before the start of the trip no hatched larvae were found. The most obvious interpretation seemed to be that the mechanical action of the sand particles had aided in the hatching of these eggs. Accordingly embryonated eggs were stirred in moist sand. "Hatching" started almost at once and most of the eggs were "hatched" in a short time. Similarly eggs could be readily "hatched" by shaking or stirring with lead shot, pebbles or glass beads. In only 10 minutes of such shaking 80 per cent of the eggs were "hatched." Treatment of infective eggs with 30 per cent antiformin for 15 minutes so weakened the shell by dissolving the outer albuminous coat that the "hatching" occurred with a very slight amount of friction. This was true of newly infective eggs or eggs which had not hatched under the various conditions tested. Living larvae were found in sand culture two weeks after it was gently stirred. However, there seemed to be a reduction in the number of eggs at the end of this time suggesting that larvae had continued to hatch for nearly two weeks rather than original larvae having lived that time. In order to test this hypothesis larvae freshly hatched from infective eggs were transferred to Ringer's solution at 37° C. All such larvae were dead by the second day.

The writer feels that the many reports of the hatching of ascaris eggs have been misleading, *i.e.,* that the "hatching" is evidently the effect of external conditions probably in most cases mechanical injury to the egg shell and should not be interpreted as biological hatching of the eggs. A suggestion of this has already been given by Itagaki (1927, World's Poultry Congress) who says that eggs in physiological salt solution or 0.2 per cent formalin never hatched at room temperature if no mechanical or physical irritation were given them. The shells may be weakened by treatment with various chemicals but there seems to be no evidence that "hatching" is stimulated. Mechanical action may even cause hatching in the lumen of the intestine (Ohba, l. c.) but certainly it is the only factor which in our experience will cause extra-corporeal hatching with any degree of regularity. Furthermore, larvae thus hatched are apparently not adapted for free living life and die quickly. It seems very unlikely that they could be seriously considered as a means of infection under natural conditions as suggested by Maplestone (1928, Ind. Med. Gaz., 65: 553–554).—ANNE MCRAE, *The Johns Hopkins University, School of Hygiene and Public Health.*

TETRAMASTIX AMBYSTOMA, A NEW SPECIES OF FLAGELLATE PROTOZOON FROM THE SALAMANDER, *AMBYSTOMA*

While studying the intestinal protozoa of reptiles and amphibians, a *Trichomonas* with three anterior flagella, *Hexamita, Prowazekella* and a quadriflagellate were encountered in the intestinal contents of an *Ambystoma.* The quadriflagellate, which is probably a new species of the genus *Tetramastix* Alexeieff, 1916, may be described as follows.

Morphology. The size is variable, some being small and others larger and broader. The size of the small form is 12 x 2 microns. The larger individuals measure 23 x 8 microns. There are also intermediate forms. The animal has a rounded anterior end and tapers posteriorly to a point. Some individuals, however, have both ends rounded. The flagella are more or less of equal length and directed anteriorly. There are two blepharoplasts, each of which serves as the point of origin of a pair of flagella. The nucleus is situated just behind the blepharoplast and contains several irregular masses of chromatin. A rhizoplast connects one of the blepharoplasts with the nucleus. In specimens fixed with Schaudinn's fluid and stained by Heidenhain's method a chromatic mass lying near the nucleus can be made out. This structure probably represents the parabasal. A very characteristic feature of the parasite is the two sets of parallel striae or ridges arranged obliquely and intersecting one another on the surface of



All figures drawn to scale (× 2000 approx.). Fig. 1. Fixation: Schaudinn's fluid; stain, Heidenhain's iron-haematoxylin. The remainder were fixed with osmic acid and absolute alcohol and stained by Giemsa's stain. All figures except no. 1 show the characteristic striae.

Description of Figures

Fig. 1. Form showing the parabasal body.
Fig. 2. Individual showing the rhizoplast and a vacuole.
Fig. 3. Form with rounded posterior end and vacuoles.
Figs. 4 & 5. Dividing stages.
Fig. 6. A slender individual.

the body. Vacuoles are usually present, but do not contain any food material, and are probably eosinophilic. There is no cytostome and consequently the ingestion of bacteria is impossible. No axostyle is present.

Multiplication takes place by longitudinal division. First the blepharoplasts divide and from the newly formed blepharoplasts young flagellar outgrowths make their appearance. This is followed by the division of the nucleus.

Cultivation. Although the flagellate remained alive for about a week in haemoglobin-saline medium, it did not multiply in this nor in any of the common media used for the cultivation of intestinal flagellates.

Diagnosis. This flagellate belongs to the group of parasitic Tetramitidae. It corresponds to a large extent with *Monocercomonas bufonis* described by Dobell (Quart. Journ. Micros. Sci., vol. 53, 201, 1909).

Alexeieff (C. R. Soc. Biol., 79: 1076, 1916) established a new genus *Tetramastix* for *Monocercomonas bufonis* and called it *Tetramastix bufonis*. *Tetramastix* differs from *Monocercomonas* in that it has no axostyle as shown by Mackinnon (Parasitology, 5: 180, 1913).

The genera *Tetramastix* and *Polymastix* have certain common characteristics, *viz.*, presence of ridges and four anteriorly directed flagella arising from two blepharoplasts. But the latter genus have a cytostome, ingest bacteria, divide by transverse constriction of the body and are parasitic in insects and myriapods. Hence, the animal now described belongs with greater probability to the genus *Tetramastix* as it has more characteristics common with that genus than with any other.

Grassé (Arch. Zool. Exp., 65: 426, 1926) has made intensive studies on *Tetramastix bufonis* but does not mention the presence of ridges on the body surface which is a constant and easily demonstrable feature of the parasite here described. Hence it is proposed to call this a new species under the name of *Tetramastix ambystomae*.—B. M. Das Gupta, *Johns Hopkins University, School of Hygiene and Public Health.*

THE NATURE OF THE "CELL BODY" OF *TRICHURIS* AND "STICHOSOME" OF *AGAMERMIS*

The significance of the cells closely associated with the posterior part of the esophagus in trichuroids and mermithoids, termed "cell body," "stichosome," and "paresophageal body," has long been a matter of conjecture. A study recently, by the writer, of well preserved material showed that each cell of the "cell body" has a separate opening into the lumen of the esophagus, these orifices alternating. Rauther (1918, Zool. Jahrb. v. 40: 441–514, pls. 20–24) observed orifices from cells of the "cell body" into the lumen in *Trichuris*, a finding which the writer (Chitwood, 1930, J. Parasitol., v. 17: 35–42, pls. 5–6) was subsequently unable to verify. It may now be said definitely that these cells are esophageal glands. In addition there are 3 (4 in 1 series of sections) large nuclei in the wall of the esophagus anterior to the cell body, which also appear to be those of esophageal glands. These observations do not validate a conception held by many nematologists that the esophagus of trichuroids consists of a series of cells, since the esophagus proper retains its identity and the gland cells outside of it are comparable to the glands lying outside of the esophagus in the body cavity of some tylenchoids.

A study of the esophagus of parasitic larval *Agamermis decaudata* showed very clearly that each "stichocyte" is likewise an esophageal gland with an opening into the lumen of the esophagus. In addition to these glands there are 3 glands opening into the lumen at the anterior end of the "stichosome." Because of the torsion of the esophagus, it is difficult in both *Trichuris* and *Agamermis* to determine the sectors of the esophagus into which these various glands open; presumably the "cell body" and "stichosome" have subventral openings. There is little doubt that the two structures are homologous, and in view of this homology "stichosome" (*στίχος*, row + *σῶμα*, body) is adopted as a more appropriate term for these structures than either "cell body" or "paresophageal body." The double row of cells (stichosome of authors) in *Agamermis* explains the alternation of orifices in the single row of cells (cell body of authors) in *Trichuris*, since the fusion of an original double row of cells would be expected to result in a single row having alternating orifices. These observations furnish additional evidence of the natural grouping of the Dorylaimoidea, Mermithoidea and Trichuroidea in the suborder Dorylaimata, for in the dorylaims (data unpublished) there are 5 esophageal glands of which 1 is dorsal and 4 are subventral, each with a separate opening near its nucleus. The mermithoids and the trichuroids, in all probability, represent a further development from certain of the dorylaims through the multiplication of the subventral esophageal glands.—B. G. Chitwood, *Zoological Divi-*

sion, Bureau of Animal Industry, U. S. Department of Agriculture, Washington, D. C.

STUDIES ON THE PATHOLOGY OF *NIPPOSTRONGYLUS MURIS* IN RATS AND MICE

That *Nippostrongylus muris* (Yokogawa, 1920) when present in large numbers is decidedly pathogenic was first noted by Africa (1931, Jour. Parasit., 18: 1–13), who reported that heavily infested animals were manifestly ill as shown by their emaciated condition. Their hair was ruffled and standing on end and their eyes dull and lusterless. These animals usually sat still and were quite apathetic to their surroundings even shunning food that was placed before them. The stools of such animals were soft and mucoid. He also reported finding the adult worms in clumps in the intestine usually pinned into the mucosa for considerable depths.

The present writer observed these same things in experimentally infected rats and mice. It was further noted that month-old rats and mice given respectively 15,000 and 3,000 infective larvae died after two days, showing severe symptoms of pneumonia. Any animals surviving the severe tissue destruction of the pulmonary migration usually survived the intestinal stages.

The macroscopical appearance of the lungs at autopsy depends upon the number of larvae migrating. In mild cases the lungs are somewhat edematous and show small bright red hemorrhagic areas in which larvae can usually be demonstrated. In severe cases the lung may be entirely hemorrhagic, congested and edematous. The bronchi sometimes contain a frothy mucous.

The microscopical pathological picture of pulmonary nippostrongyliasis shows a type of lobar pneumonia somewhat similar to that produced by ascaris larvae. Portions of the sections showed areas of marked consolidation and diffuse hemorrhage. In the areas in proximity to the migrating larvae a compensating emphysema may be seen (Figs. 1 and 2). These and the other photomicrographs were made with the aid of Mr. K. B. Kerr from sections fixed in Bouin's and stained with hematoxylin and eosin.

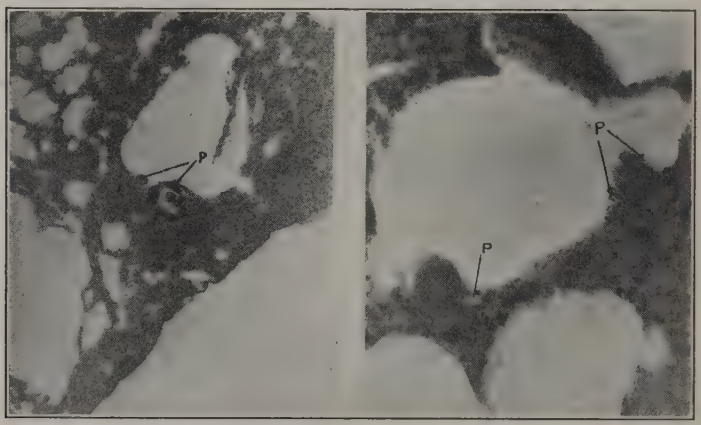


Fig. 1. (Left) Larvae breaking into alveoli. Note pigment *P* contained within the larvae. 72 hours after infection. ×80.

Fig. 2. (Right) Showing pigment *P* near cross section of larvae. ×344.

Deposits of pigment usually free but sometimes within mononuclear leucocytes were found in the tissue particularly near the larvae. This pigment ranged from ochreous or golden brown to black in color and in several instances was found contained within the digestive tract of the larvae (Figs. 1 and 2). A substance indistinguishable from "malarial pigment" by physico-chemical tests has been reported by Fairley (1920, Jour. Path. and Bact. 23: 287–314) as occurring in the livers of monkeys infected with *Schistosoma hematobium* and *S. mansoni*. The intestinal caeca of the schistosomes also contained an abundance of this pigment which was thought to be derived from blood metabolized in the process of digestion. The pigment occurring in the larvae of *N. muris* and in infected mouse lungs gave a negative test for iron (absence of Prussian blue when treated with potassium ferrocyanide) and was soluble in 5 per cent nitric acid alcohol. This test in addition to its physical appearance suggests that it may be of the nature of the "Bilharzial pigment" described by Fairley. Inasmuch as the pigment is found inside the larvae it is possible that the pigment deposited outside may be excreted metabolic waste from the larvae. Whether it might arise externally as the result of some hemolytic or proteolytic action of a larval toxin or be of some other origin is not known.

The adult worms were usually found in clumps or nests in the anterior half of the intestine especially in the younger rats or mice or in cases of heavy infection. In older animals of both species the worms tended to be more scattered, perhaps due to the factor of age resistance. The worms nests formed bright red patches on the intestinal wall readily seen from the outside. This was first thought to be due to the red color of the worms but closer examination showed the villi in these worm nests to be greatly distended and red in color due to the excess amount of blood in the dilated capillaries of each villus (Figs. 3 and 4).

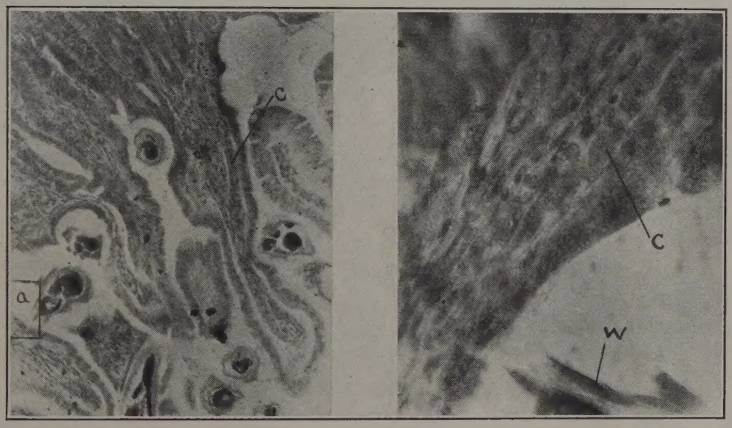


Fig. 3. (Left) Section of rat's intestine 14 days after infection showing adult worms among the villi. Note dilation of capillary *C* and local displacement of tissue near the worms. ×80.

Fig. 4. (Right) High magnification of area *a* from Fig. 3, showing blood in dilated capillary of villus near worm *W*. ×344.

That the adults migrated deeply among the villi was evident from macroscopical examination. By fixing sections of intestine containing these nests in Bouin's killing fluid as soon as the animal was dead it was possible to obtain sections with the adults still in place.

Microscopically it was observed that the worms migrate extensively in and out among the villi causing local destruction and shrinking of the tissue (Fig. 3). Somewhat similar lesions in the stomach of cats caused by *Ollulanus tricuspis*, a trichostrongyle closely related to *Nippostrongylus*, have been described by Cameron (1932, Jour. Helm., 10: 231–234). A hint that *Nippostrongylus* may feed upon the glandular secretions or tissues of the host is shown in Fig. 5. This section shows the anterior end of an adult that has penetrated between two villi destroying the glandular tissue in the mucosa and the submucosa as far as the circular muscle coat of the intestine. A section of a rat's intestine (Fig. 6) shows

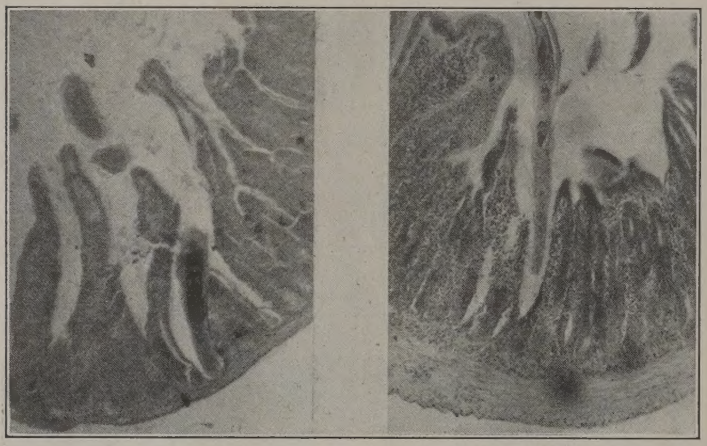


Fig. 5. (Left) Cross section of mouse intestine 10 days after infection showing anterior end of adult worm that has penetrated to the muscular wall of the intestine. ×80.

Fig. 6. (Right) Adult worm penetrated between villi of rat's intestine 12 days after infection. ×80.

a worm in somewhat the same position that has caused destruction of the villus epithelium. This type of adult migration suggests the possibility that intestinal perforation with its complications may occur. From the evidence here presented it seems safe to conclude that this parasite is decidedly pathogenic to both rats and mice especially when present in large numbers.—DALE A. PORTER, *Department of Helminthology, School of Hygiene and Public Health, Johns Hopkins University.*

REVIEWS

THE INTESTINAL PARASITES OF DOMESTICATED ANIMALS. A Manual for Veterinary Surgeons. By Thomas W. M. Cameron. A. and C. Black, Lt., London, xii, 292 pp., 1934.

This very readable textbook of parasitology should be an invaluable aid to students and practitioners of veterinary medicine. It is not, and is not intended to be, a check list of all the internal parasites of domesticated animals but is a rather concise treatise on those forms more likely to come to the attention of the practicing veterinarian. The book is divided into seven sections, the first of which (9 pp.) is given over to a brief general introduction including a definition of the scope of the subject of parasitology, an explanation of the binomial system of nomenclature, and a historical review of the development of the subject. The second section (25 pp.) covers the protozoa, including a description of the organisms, their life histories, geographical distribution, diagnosis and control with an account of the symptomatology, pathology and treatment of the diseases they produce. The classification and synonymy, particularly in the Trypanosomidae would not meet with universal approval but is understandable and offers a good working basis for the student or practitioner. The third section (169 pp.) covers the helminths. The classification is conservative for the most part but the choice of generic and specific names does not always conform to the usually accepted usage. Pathology, symptomatology, treatment and control are often discussed for groups of related forms which permits a comparative and more adequate presentation of the information available. In the end of the section the geographical distribution of the helminths is summarized. In sections IV (20 pp.), V (8 pp.), and VI (23 pp.) immunology and serology, anthelmintics and parasitological techniques are respectively treated. Although these sections are short the material in them is well selected and effectively handled. A tabulation is included in section V of the uses together with dosages of the more common anthelmintics. In the last section are included the host lists of the parasites covered and a classified list of references to the more important monographs and reference books.

Although many of the generalizations made throughout the book are, the reviewer believes, based on too few data yet it is evident that the author by such generalizations has brought this specialized subject with a reasonable degree of accuracy more clearly than heretofore within the reach of the general practitioner. He is to be complimented on his selection and handling of material and on the valuable collection of illustrations he has included.—G. F. OTTO.

COCCIDIA AND COCCIDIOSIS OF DOMESTICATED, GAME AND LABORATORY ANIMALS AND OF MAN. By Elery R. Becker. Collegiate Press, Ames, Iowa, IX + 147 pp., 1934.

Despite the frequence, wide distribution and significant relations of the Coccidia, very little attention has been paid to the group. Even specialists endeavoring to follow up the matter find references widely scattered in the literature and unfortunately often inaccessible. Doctor Becker has done a good service in writing this book for the use of all interested, either superficially or intensively, in the topic.

The work starts with a general account of the structural and biological features of the group, in which facts of importance from widely diverse sources have been brought together in brief and well balanced form. Then follow some twenty chapters dealing succinctly with the parasites of this group found in individual hosts among mammals and birds, together with an indication of the diseases induced by those species. Further subdivisions of the work contain comments on species not handled in the text, a complete host catalogue, notes on technique, a

fine bibliography and indices. The work is well illustrated by plates and text figures, in considerable part representing the investigations of the author. The book, which has been put out by the Collegiate Press of Ames, Iowa, is well printed. Great praise is due the author for his admirable restraint in avoiding the temptation to produce a monograph and for furnishing such a fine, well digested summary of present day knowledge of the group within limits which permit of ready use by those busily occupied with duties in other fields while at the same time the presentation is thoroughly accurate to serve the purpose of laboratory workers and even of those engaged in researches on members of the group.—H. B. WARD.